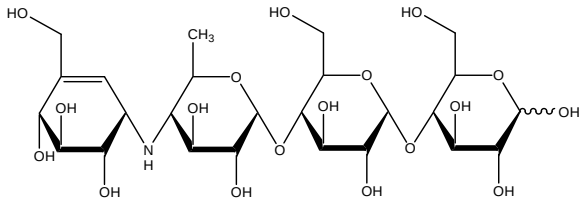
Monographs, Part I

Acarbose



$C_{25}H_{43}NO_{18}$: 645.60

(2*R*,3*R*,4*R*,5*S*,6*R*)-5-{[(2*R*,3*R*,4*R*,5*S*,6*R*)-5-{[(2*R*,3*R*,4*S*,5*S*,6*R)*-3,4-dihydroxy-6-methyl-5-{[(1*S*,4*S*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}oxan-2-yl]oxy}-3,4-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-6-(hydroxymethyl)oxane-2,3,4-triol
[*56180-94-0*]

Acarbose is produced by certain strains of *Actinoplanes utahensis*.
Acarbose contains not less than 95.0 % and not more than 102.0 % of acarbose ($C_{25}H_{43}NO_{18}$), calculated on the anhydrous basis..

Description Acarbose appears as white or yellowish amorphous powder.
Acarbose is hygroscopic.
Acarbose is very soluble in water, soluble in methanol and practically insoluble in dichloromethane.

Identification (1) Determine the infrared spectra of Acarbose and Acarbose RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Specific Optical Rotation $[\alpha]_D^{20}$: +168 ~ +183° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving Acarbose in water so that each mL contains 50 mg is between 5.5 and 7.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Acarbose according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of the standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately 0.2 g of Acarbose, dissolve in water to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine A_i, the peak area of each related substance obtained from the test solution, and A_S, the peak area of acarbos obtained from the standard solution, and calculate the amount of percentage of each related substances: the amount of related substance I {*O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose} is not more than 0.6 %; the amount of related substance II {(1*R*,4*R*,5*I*,6*R*)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside} is not more than 0.5 %; the amount of related substance III {α-D-Glucopyranosyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside} is not more than 1.5 %; the amount of related substance IV {4-*O*-[4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl) cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-D-glucopyranose} is not more than 1.0 %; the amount of related substance V {*O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*, 6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose (4-*O*-α-acarbosyl-D-fructopyranose)} is not more than 0.2 %; the amount of related substance VI {*O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl) cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose (4-*O*-α-acarbosyl-D-glucopyranose)} is not more than 0.3 %; the amount of related substance VII {α-D-Glucopyranosyl *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranoside(α-D-glucopyranosyl α-acarboside)} is not more than 0.3 %; the amount of related substance VIII {*O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl) cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose} is not more than 0.2 %. the amount of each other related substance is not more than 0.2 % and the total amout of related substances is not more than 3.0 %. For these calculations, use the peak areas of related substance II, IV, V, VI, and VII determined by the automatic integration method, after multiplying by their relative response factor, 1.6, 1.33. 0.8, and 0.8, respectively

$$\text{Amount (\%) of each related substance} = \frac{A_i}{A_S}$$

System suitability
Relative retention time: Relative retention time

of related substance I, II, III, IV, V, VI, VII, and VIII are 0.9, 0.8, 1.2, 0.5, 1.7, 1.9, 2.2, and 0.6, respectively.

Water Not more than 4.0 % (0.300 g, coulometric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1.0 g).

Assay Weigh accurately about 200 mg of Acarbose, dissolve in water to make 10 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Acarbose RS, add 5 mL of water, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of acarbose in each solution.

$$\text{Amount (mg) of acarbose } (C_{25}H_{43}NO_{18}) = \frac{A_T}{A_S} \times C \times 10$$

C: Concentration (mg/mL) of acarbose in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.0 mm in internal diameter and about 25 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

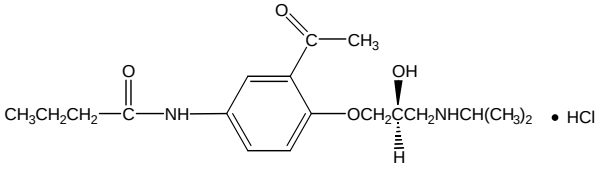
Mobile phase: A mixture of acetonitrile and phosphate buffer (750 : 250).

Flow rate: 1.0 mL minute.

Phosphate buffer—Dissolve 0.6 g of monobasic potassium phosphate and 0.35 g of dibasic sodium phosphate in 900 mL of water, and add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Acebutolol Hydrochloride



and enantiomer

$C_{18}H_{28}N_2O_4 \cdot HCl$: 372.89

N-[3-Acetyl-4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]butanamide monohydro-chloride [*34381-68-5*]

Acebutolol Hydrochloride, when dried, contains not less than 98.0 % and not more than 102.0 % of acebutolol hydrochloride ($C_{18}H_{28}N_2O_4 \cdot HCl$).

Description Acebutolol Hydrochloride appears as white to pale yellowish white crystals or crystalline powder.

Acebutolol Hydrochloride is freely soluble in water, in methanol, in ethanol (95) or in acetic acid (100) and practically insoluble in ether.

A solution of Acebutolol Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Acebutolol Hydrochloride and Acebutolol Hydrochloride RS, in 0.01mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Acebutolol Hydrochloride and Acebutolol Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Acebutolol Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Melting Point 141 ~ 145 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Acebutolol Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Acebutolol Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 40 mg of Acebutolol Hydrochloride in 2 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add methanol to make exactly 25 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5 : 4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm). Any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 3 hours).

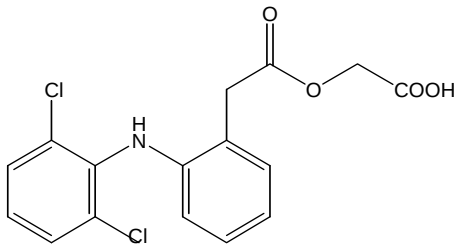
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.25 g of Acebutolol Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.289 mg of $C_{18}H_{28}N_2O_4 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Aceclofenac



$C_{16}H_{13}Cl_2NO_4$: 354.19

2-{2-[2-(2,6-Dichloroanilino)phenyl]acetyl}oxyacetic acid [*89796-99-6*]

Aceclofenac, when dried, contains not less than 99.0 % and not more than 101.0 % of aceclofenac ($C_{16}H_{13}Cl_2NO_4$).

Description Aceclofenac is a white crystalline powder.
Aceclofenac is freely soluble in ammonium peroxydisulfate or in acetone, soluble in methanol or in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 10 mg of Aceclofenac in 10 mL of ethanol (95), and to 1 mL of this solution, add 0.2 mL of a mixture of equal volume of a solution of potassium ferricyamide (6 in 1000) and a solution of iron (III) chloride (9 in 1000). Allow to stand in the dark for 5 minutes, add 3 mL of a solution of hydrochloric acid (10 in 1000), and allow to stand in the dark for 15 minutes again: A blue color develops and a precipitate is formed.

(2) Dissolve 50 mg of Aceclofenac in 100 mL of methanol, and to 2 mL of this solution, add methanol to make 50 mL, and determine the absorption spectrum of this solution between 220 nm and 370 nm as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum at 275 nm. The specific absorbance at the maximum is 320 to 350.

(3) Determine the infrared spectra of Aceclofenac and Aceclofenac RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Aceclofenac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.1 g of Aceclofenac, accurately weighed, in 50 mL of the mobile phase, and use this solution as the test solution. Dissolve diclofenac sodium RS, equivalent to 5 mg of diclofenac, in 50 mL of the mobile phase, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make 50 mL, and use this solution as the standard solution (2). Pipet 5 mL of the standard solution (1), add 0.25 mL of the test solution, add the mobile phase to make 50 mL, and use this solution as the standard solution (3). Perform the test with 10 μL each of the test solution and the standard solution (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions: the area of each peak other than the peak of Aceclofenac from the test solution is not larger than the peak area of Aceclofenac from the standard solution (2) (not more than 0.2 %), and the total area of each peak other than the peak of Aceclofenac from the test solution is not more than 2.5 times of the peak area of Aceclofenac from the standard solution (2) (not more than 0.5 %). Omit the peak from the test solution, which peak area is less than 0.2 times of the peak area of the main peak from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of mixture of a solution of acetic acid (100) (12 in 10000), acetonitrile, and tetrahydrofuran (550 : 225 : 225) to 3.5 with sodium hydroxide TS.

Flow rate: 1 mL/minute

System suitability:

Detection sensitivity: Adjust the detection sensitivity so that the peak height of Aceclofenac and diclofenac obtained from the standard solution (3) composes 50 % of the full scale.

System performance: When the procedure is run with 10 μL of the standard solution (3) under the above operating conditions, Aceclofenac and diclofenac are eluted in this order with the resolution between their peaks being not less than 8.0.

Time span of measurement: About 10 times as long as the retention time of Aceclofenac.

Loss on Drying Not more than 0.5 % (1.0 g, between 100 ºC and 105 ºC)

Residue on Ignition Not more than 0.1 % (1.0 g).

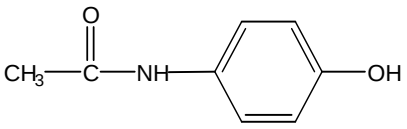
Assay Weigh accurately about 0.3 g of Aceclofenac, previously dried, dissolve in exact 40 mL of methanol, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 35.419 mg of $C_{16}H_{13}Cl_2NO_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Acetaminophen



Paracetamol $C_8H_9NO_2$: 151.16

N-(4-Hydroxyphenyl)acetamide [*103-90-2*]

Acetaminophen, when dried, contains not less than 98.0 % and not more than 101.0 % of acetaminophen ($C_8H_9NO_2$).

Description Acetaminophen appears as white crystals or crystalline powder.
Acetaminophen is freely soluble in methanol or in ethanol (95), sparingly soluble in water, and very slightly soluble in ether.
Acetaminophen dissolves in sodium hydroxide TS.

Identification Determine the infrared spectra of Acetaminophen and Acetaminophen RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 169 ~ 172 ºC.

Purity (1) ***Chloride***—Dissolve 4.0 g of Acetaminophen in 100 mL of water by heating, cool with shaking in ice-water, allow to stand until ordinary temperature is attained, add water to make 100 mL and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(2) ***Sulfate***—To 25 mL of the filtrate, obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Acetaminophen according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Acetaminophen according to Method 3 and perform the test using Apparatus B (not more than 2 ppm).

(5) ***Related substances***—Dissolve 50 mg of Acetaminophen in 1 mL of methanol, add the mobile phase to make 50 mL and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the peak area of Acetaminophen from the test solution is not larger than the peak area of Acetaminophen from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of 0.05 mol/L monobasic potassium phosphate TS, pH 4.7 and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of Acetaminophen is about 5 minutes.

System Suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of Acetaminophen obtained from 10 μL of the standard solution is about 15 % of the full scale.

System performance: Dissolve 10 mg each of Acetaminophen and *p*-aminophenol in 1 mL of methanol, add the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions. *p*-Aminophenol and Acetaminophen are eluted in this order with the resolution between their peaks being not less than 7.

Time span of measurement: About 6 times as long as the retention time of Acetaminophen after the solvent peak.

Loss on Drying Not more than 0.3 % (0.5 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 20 mg each of Acetaminophen and Acetaminophen RS, previously dried, dissolve in 2 mL of methanol and add water to make exactly 100 mL. Pipet 3 mL each of these solutions, add water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbance, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorption at about 244 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank.

$$\text{Amount (mg) of acetaminophen } (C_8H_9NO_2) = \text{Amount (mg) of Acetaminophen RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Acetaminophen Tablets

Paracetamol Tablets

Acetaminophen Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of acetaminophen ($C_8H_9NO_2$: 151.17).

Method of Preparation Prepare as directed under Tablets, with Acetaminophen.

Identification (1) The retention time of main peak of the test solution and the standard solution for Assay is the same.

(2) Weigh a portion of powdered Acetaminophen Tablets, equivalent to 50 mg of Acetaminophen according to labeled amount, add 50 mL of methanol and mix, filter and use this filtrate as the test solution. Separately, weigh 5 mg of Acetaminophen RS, add methanol to make 5 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of dichloromethane and methanol (4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm). The R_f values of the spots from the test solution and the standard solution are the same.

Dissolution Test Perform the test with 1 tablet of Acetaminophen Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of diluted phosphate buffer solution, pH 5.8, as the dissolution solution. Take the dissolution solution 30 minutes after starting the test, make any necessary dilution of the filtrate by adding the dissolution solution and use this solution as the test solution. Separately, weigh accurately about sufficient quantity of Acetaminophen RS, previously dried in a dessicator (silica gel for 18 hours) and dissolve in the test solution, to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 243 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Acetaminophen Tablets in 30 minutes should be not less than 80 %.

Phosphate buffer solution, pH 5.8—To 50 mL of 0.2 mol/L dibasic potassium phosphate TS, add 3.6 mL of 0.2 mol/L of sodium hydroxide TS and add water to make 200 mL.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Acetaminophen Tablets. Weigh accurately equivalent to about 0.1 g of Acetaminophen, add 100 mL of mobile phase, shake for 10 minutes, then shake strongly for 5 minutes, add mobile phase to make 200 mL, pipet 5 mL of this solution, add mobile phase to make 250 mL and filter through a membrane filter with a pore size of not more than 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 20 mg of Acetaminophen RS, previously dried in 105 ºC for 2 hours, dissolves in mobile phase to make exactly 100 mL, pipet 5 mL of this solution, add mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine, A_T and A_S, of the peak area of acetaminophen in each solution.

$$\text{Amount (mg) of acetaminophen } (C_8H_9NO_2) = \text{Amount (mg) of Acetaminophen RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 243 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of water and methanol (3 : 1).

Flow rate: 1.5 mL/minute.

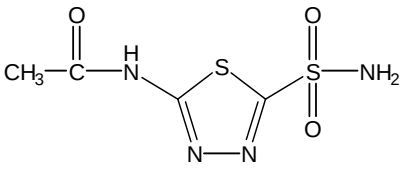
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating condition, the symmetry factor of the peak of acetaminphen is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Acetazolamide



$C_4H_6N_4O_3S_2$: 222.25

5-Acetamido-1,3,4-thiadiazole-2-sulfonamide [*59-66-5*]

Acetazolamide contains not less than 98.0 % and not more than 102.0 % of acetazolamide ($C_4H_6N_4O_3S_2$), calculated on the dried basis.

Description Acetazolamide is a white to pale yellowish white, crystalline powder, is odorless and has a slight bitter taste.

Acetazolamide is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in ether.

Melting point—About 255 ºC (with decomposition)

Identification (1) To 0.1 g of Acetazolamide, add 5 mL of sodium hydroxide TS, then add 5 mL of a solution of 0.1 g of hydroxylamine hydrochloride and 50 mg of cupric sulfate in 10 mL of water: a pale yellow color develops. Then heat this solution for 5 minutes: a deep yellow color is produced gradually.

(2) To 20 mg of Acetazolamide, add 2 mL of dilute hydrochloric acid, boil for 10 minutes, cool and add 8 mL of water: this solution responds to the Qualitative Tests for primary aromatic amines.

(3) To 0.2 g of Acetazolamide, add 0.5 g of granulated zinc and 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moistened lead acetate paper.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Acetazolamide in 10 mL of sodium hydroxide TS: the solution is clear and colorless to pale yellow.

(2) ***Chloride***—To 1.5 g of Acetazolamide, add 75 mL of water and warm at 70 ºC for 20 minutes with occasional shaking. After cooling, filter and to 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(3) ***Sulfate***—To 25 mL of the filtrate obtained in (2), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Acetazolamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Silver-reducing substances***—Wet 5 g of Acetazolamide with 5 mL of aldehyde-free ethanol and add 125 mL of water, 10 mL of nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS. Stir for 30 minutes while protected from light, filter through a glass filter (G3) and wash the residue on the glass filter with two 10 mL volumes of water. Combine the filtrate with the washings, to the solution, add 5 mL of ammonium iron (III) sulfate TS and titrate with 0.1 mol/L ammonium thiocyanate VS: not less than 4.8 mL of 0.1 mol/L ammonium thiocyanate VS is consumed.

(6) ***Selenium***—Proceed with 0.2 g of Acetazolamide as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1000 mL combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 with diluted ammonia solution (28) (1 in 2), dilute with water to make 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(7) ***Related substances***—Dissolve 100 mg of Acetazolamide in a mixture of acetone and methanol (1 : 1)

to make 10 mL, and use this solution as the test solution. To 2 mL of the test solution add a mixture of acetone and methanol (1 : 1) to make 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and 1 mol/L ammonia water (88 : 12) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the standard solution (not more than 2.0 %).

Loss on Drying Not more than 0.5 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

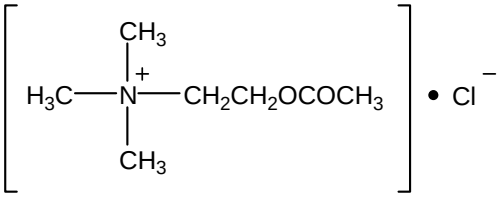
Assay Weigh accurately about 0.15 g of Acetazolamide and dissolve in 400 mL of water in a water-bath. After cooling, add water to make exactly 1000 mL. Pipet 5 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS and then add water to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 265 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of acetazolamide } (C_4H_6N_4O_3S_2) = \frac{A}{474} \times 200000$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Acetylcholine Chloride for Injection



$C_7H_{16}ClNO_2$: 181.66

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use. Acetylcholine Chloride for Injection contains not less than 98.0 % and not more than 102.0 % of acetylcholine chloride ($C_7H_{16}ClNO_2$) and not less than 19.3 % and not more than 19.8 % of chlorine (Cl: 35.45), calculated on the dried basis. Acetylcholine Chloride for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

Method of Preparation Prepare as directed under Injections.

Description Acetylcholine Chloride for Injection appears as white crystals or crystalline powder.
Acetylcholine Chloride for Injection is very soluble in water, freely soluble in ethanol (95).
Acetylcholine Chloride for Injection is extremely hygroscopic.

Identification (1) Determine the infrared spectra of Acetylcholine Chloride for Injection and Acetylcholine Chloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Acetylcholine Chloride for Injection in water (1 in 10) responds to the Qualitative Tests (2) for chloride.

Melting Point 149 ~ 152 °C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point determination immediately after drying both the sample and the tube at 105 °C for 3 hours and determine the melting point.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of water: the solution is clear and colorless.

(2) ***Acidity***—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of freshly boiled and cooled water, and add 1 drop of bromthymol blue TS, and use this solution as the test solution. To the test solution add 0.30 mL of 0.01 mol/L sodium hydroxide VS: the solution is blue in color.

(3) ***Heavy metals***—Proceed with 2.0 g of Acetylcholine Chloride for Injection according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay (1) ***Acetylcholine chloride***—Weigh accurately the contents of not less than 10 Acetylcholine Chloride for Injections. Weigh accurately about 0.5 g of the contents, dissolve in 15 mL of water, then add exactly 40 mL of 0.1 mol/L sodium hydroxide VS, stopper loosely and heat on a water-bath for 30 minutes. Cool quickly and titrate the excess sodium hydroxide with 0.05 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

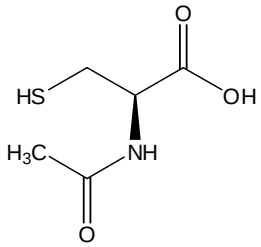
Each mL of 0.1 mol/L sodium hydroxide VS
= 18.166 mg of $C_7H_{16}ClNO_2$

(2) ***Chlorine***—Titrate the solution, which has been titrated in (1), with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 3.5453 mg of Cl

Containers and Storage ***Containers***—Hermetic containers.

Acetylcysteine



$C_5H_9NO_3S$: 163.20

(2*R*)-2-Acetamido-3-sulfanylpropanoic acid [*616-91-1*]

Acetylcysteine, when dried, contains not less than 98.0 % and not more than 102.0 % of acetylcysteine ($C_5H_9NO_3S$).

Description Acetylcysteine is a white, crystalline powder.
Acetylcysteine is freely soluble in water or ethanol (95), and practically insoluble in dichloromethane.

Identification (1) Determine the infrared spectra of Acetylcysteine and Acetylcysteine RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +21 ~ +27°. Weigh accurately about 1.25 g of Acetylcysteine, dissolve in 1 mL of disodium ethylenediaminetetraacetate solution (1 in 100) and 7.5 mL of sodium hydroxide solution (1 in 25), and add phosphate buffer solution, pH 7.0, to make 25 mL, and determine the optical rotation of this solution.

pH 7.0 phosphate buffer solution—Add 50 mL of 1 mol/L monobasic potassium phosphate TS and 29.5 mL of 1 mol/L sodium hydroxide solution to a volumetric flask, adjust to a pH of 7.0 by adding water, and add water again to make 100 mL.

pH Dissolve 1.0 g of Acetylcysteine in 100 mL of freshly boiled and cooled water: the pH of this solution is between 2.0 and 2.8.

Purity ***Heavy metals***—Proceed with 2.0 g of Acetylcysteine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of lead standard solution (not more than 10 ppm).

Loss on Drying Not more than 0.1 % (1 g, in vacuum at the pressure not exceeding 0.67 kPa, 70 ºC, 4 hours).

Residue on Ignition Not more than 0.5 % (2 g, 600 ºC).

Assay Weigh accurately about 1.0 g of Acetylcysteine, previously dried, dissolve in freshly prepared sodium metabisulfite solution (1 in 2000) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add sodium metabisulfite solution (1 in 2000) to make exactly 200 mL, and use this solution as the test solution. Separately, dissolve about 1.0 g of Acetylcysteine RS, accurately weighed, in sodium metabisulfite solution (1 in 2000) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add sodium metabisulfite solution (1 in 2000) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of Acetylcysteine to that of the internal standard, respectively.

$$\text{Amount (mg) of Acetylcysteine } (C_5H_9NO_3S) = \text{Amount (mg) of Acetylcysteine RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve of 1 g of dl-phenylalanine in freshly prepared sodium metabisulfite solution (1 in 2000) to make 200 mL.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A solution of potassium dihydrogen phosphate, pH 3.0 (6.8 in 1000).

Flow rate: 1.5 mL/minute.

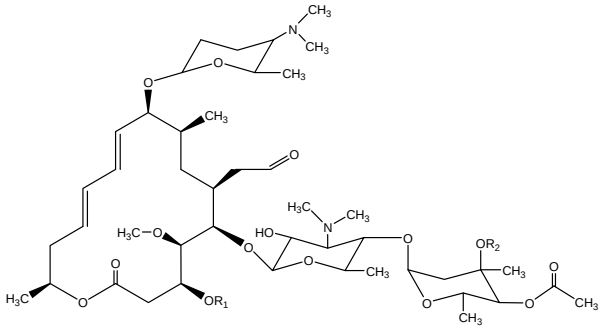
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, acetylcystein and *dl*-phenylalanine are eluted in this order with the resolution between their peaks being not less than 6.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Acetylspiramycin



Acetylspiramycin II: R_1=$COCH_3$ R_2=H
$C_{47}H_{78}N_2O_{16}$: 927.13

Acetylspiramycin III: R_2=$COCH_2CH_3$ R_2=H
$C_{48}H_{80}N_2O_{16}$: 941.16

Acetylspiramycin IV: R_1=$COCH_3$ R_2=$COCH_3$
$C_{49}H_{80}N_2O_{17}$: 969.17

Acetylspiramycin V: R_1=$COCH_2CH_3$ R_2=$COCH_3$
$C_{50}H_{82}N_2O_{17}$: 983.19

Acetylspiramycin VI: R_1=H R_2=H
$C_{45}H_{76}N_2O_1$: 885.09

acetylspiramycin II:
(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-Acetyloxy-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(4-O-acetyl-6-deoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(di-methylamino)-α-D-glucopyranoside

acetylspiramycin III:
(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(Dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)-4-(propanoyloxy)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(4-O-acetyl-6-deoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

acetylspiramycin IV:
(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-Acetyloxy-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(3,4-di-O-acetyl-6-deoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

acetylspiramycin V:
(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(Dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)-4-(propanoyloxy)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(3,4-di-O-acetyl-6-deoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

acetylspiramycin VI:
(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(Dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-4-hydroxy-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(4-O-acetyl-6-deoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

Acetylspiramycin is the derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambofaciens*.
Acetylspiramycin contains not less than 900 μg (potency) and not more than 1450 μg (potency) per mg, calculated on the dried basis. The potency of Acetylspiramycin expresses the amount of Acetylspiramycin II ($C_{47}H_{78}N_2O_{16}$: 927.13) as the mass (potency) of acetylspiramycin, and 1 mg (potency) of acetylspiramycin corresponds to 0.7225 mg of acetylspiramycin II ($C_{47}H_{78}N_2O_{16}$).

Description Acetylspiramycin appears as white to pale yellowish white powder.
Acetylspiramycin is very soluble in methanol or in acetonitrile, freely soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Acetylspiramycin and Acetylspiramycin RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Acetylspiramycin and Acetylspiramycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Content Ratio Dissolve 25 mg of Acetylspiramycin in 25 mL of the mobile phase, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_{II}, A_{III}, A_{IV}, A_V, A_{VI}, and A_{VII}, of acetylspiramycin II, acetylspiramycin III, acetylspiramycin IV, acetylspiramycin V, acetylspiramycin VI, and acetylspiramycin VII by the automatic integration method. Calculate the ratios of A_{II}, A_{IV}, and the sum of A_{III} and A_V to the sum of the above peak areas: A_{II} is between 30 and 45 %, A_{IV} is between 30 and 45 %, and the sum of A_{III} and A_V is not more than 25 %. The relative retention times of acetylspiramycin III, acetylspiramycin IV, acetylspiramycin V, acetylspiramycin VI, and acetylspiramycin VII are 1.3, 1.7, 2.3, 0.85, and 1.4, respectively, with respect to acetylspiramycin II.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 231 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS, and a solution of dipotassium hydrogen phosphate (87 in 25000) (26 : 7 : 7)

Flow rate: Adjust the flow rate so that the retention time of acetylspiramycin II is about 10 minutes.

System suitability

System performance: Dissolve 25 mg of Acetylspiramycin II RS in the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of acetylspiramycin II are 14500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak areas of acetylspiramycin II is not more than 2.0 %.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Acetylspiramycin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Proceed with 2.0 g of Acetylspiramycin according to Method 3, and perform the test (not more than 1 ppm).

Loss on Drying Not more than 3.0 % (1 g, in vacuum, P_2O_5, 60 °C, 3 hours)

Residue on Ignition Not more than 0.5 % (1 g)

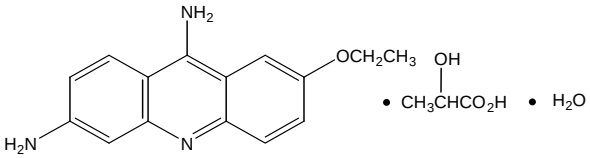
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately an amount of Acetylspiramycin, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 50 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Weigh accurately about 50 mg (potency) of Acetylspiramycin II RS, dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 3 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Acrinol Hydrate



Ethacridine Lactat $C_{15}H_{15}N_3O{\cdot}C_3H_6O_3{\cdot}H_2O$: 361.39

7-Ethoxyacridine-3,9-diamine 2-hydroxypropanoate monohydrate [*1837-57-6*]

Acrinol Hydrate, contains not less than 98.5 % and not more than 101.0 % of acrinol ($C_{15}H_{15}N_3O{\cdot}C_3H_6O_3$: 343.38), calculated on the anhydrous basis.

Description Acrinol Hydrate is a yellow, crystalline powder.

Acrinol Hydrate is sparingly soluble in water, methanol, or in ethanol (99.5).

pH—The pH of an aqueous solution of Acrinol Hydrate (1 in 100) is between 5.5 and 7.0.

Melting point—About 245 ºC (with decomposition)

Identification (1) Determine the absorption spectra

of solutions of Acrinol Hydrate and Acrinol Hydrate RS, in water (3 in 250000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Acrinol Hydrate and the Acrinol Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) To 5 mL of a solution of Acrinol Hydrate in water (1 in 100), add 5 mL of dilute sulfuric acid, shake well, allow to stand for about 10 minutes at room temperature and filter: the filtrate responds to the Qualitative Tests for lactate.

Purity (1) ***Chloride***—Dissolve 1.0 g of Acrinol Hydrate in 80 mL of water by warming in a water-bath, cool and add 10 mL of sodium hydroxide TS and water to make 100 mL. Shake well, allow to stand for 30 minutes, filter, to 40 mL of the filtrate, add 7 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare 50 mL of the control solution with 4 mL of sodium hydroxide TS, 7 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS and water (not more than 0.026 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Acrinol Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Volatile fatty acids***—Dissolve 0.5 g of Acrinol Hydrate in a mixture of 20 mL of water and 5 mL of dilute sulfuric acid, shake well, filter and heat the filtrate: no odor of volatile fatty acids is perceptible.

(4) ***Related substances***—Dissolve 10 mg of Acrinol Hydrate in 25 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 10 μL each of the test solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of any peak other than acrinol obtained with the test solution is not larger than 3 times the peak area of acrinol obtained with the standard solution (2), and the total area of all peaks other than acrinol obtained with the test solution is not larger than the peak area of acrinol with the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 2.8 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for Liquid Chromatography, and add 1.0 g of sodium 1-octanesulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of acrinol is about 15 minutes.

System suitability

Test for required detectability: Confirm that the peak area of acrinol obtained with 10 μL of the standard solution (2) is equivalent to 7 to 13 % of that with 10 μL of the standard solution (1).

System performance: When the procedure is run with 10 μL of the standard solution (2) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acrinol are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of acrinol is not more than 1.5 %.

Time span of measurement: About 3 times as long as the retention time of acrinol beginning after the solvent peak.

Water 4.5 ~ 5.5 % (0.2 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (1 g).

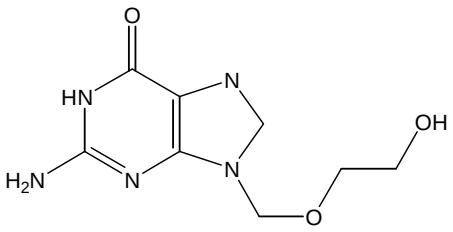
Assay Weigh accurately about 0.27 g of Acrinol Hydrate, dissolve in 5 mL of formic acid, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (1 : 1), and titrate immediately with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.34 mg of acrinol ($C_{15}H_{15}N_3O \cdot C_3H_6O_3$)

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Acyclovir



$C_8H_{11}N_5O_3$: 225.21

2-Amino-1,9-dihydro-9-((2-hydroxyethoxy)methyl)-6*H*-purin-6-one [*59277-89-3*]

Acyclovir contains not less than 98.0 % and not more than 101.0 % of acyclovir ($C_8H_{11}N_5O_3$), calculated on the anhydrous basis.

Description Acyclovir is a white crystalline powder.
Acyclovir is freely soluble in dimethylsulfoxide, slightly soluble in water, and very slightly soluble in ethanol (95).
Acyclovir dissolves in dilute hydrochloric acid.

Identification (1) Determine the infrared spectra of Acyclovir and Acyclovir RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.
(2) The retention time of main peak of the test solution for Assay and the standard solution is the same.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Acyclovir in 20 mL of dilute sodium hydroxide TS: the solution is clear and has no more color than the following control solution.

Control solution—To 2.5 mL of Color Matching Fluid F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

(2) ***Heavy metals***—Proceed with 1.0 g of Acyclovir according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Use the test solution obtained in the Assay as the test solution. Separately, weigh accurately about 25 mg of Guanine RS, dissolve in 50 mL of dilute sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the amount of guanine from the peak areas, A_T and A_S, of guanine in each solution by the following equation: not more than 0.7 %. Determine each peak area of the test solution by the automatic integration method, and calculate the amount of each related substance other than acyclovir and guanine by the area percentage method: not more than 0.2 %. The sum of the amount of guanine and the amount of each related substance calculated by the area percentage method is not more than 1.5 %.

$$\text{Amount (\%) of guanine} = \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times \frac{2}{5}$$

W_S: Amount (mg) of Guanine RS taken
W_T: Amount (mg) of Acyclovir taken

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
System suitability
System performance: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of acyclovir obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acyclovir is not more than 2.0 %.
Time span of measurement: About 8 times as long as the retention time of acyclovir beginning after the solvent peak.

Water Not more than 6.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1.0 g).

Assay Weigh accurately about 20 mg each of Acyclovir and Acyclovir RS (previously determine the water in the same manner as Acyclovir), dissolve each in 1 mL of dilute sodium hydroxide TS, add the mobile phase to make exactly 20 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of acyclovir in each solution.

$$\text{Amount (mg) of acyclovir } (C_8H_{11}N_5O_3) = \text{Amount (mg) of Acyclovir RS,}$$

$$\text{calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength; 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 20 °C

Mobile phase: Dissolve 1.0 g of sodium 1-decanesulfonate and 6.0 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To this solution add 40 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of acyclovir is about 3 minutes.

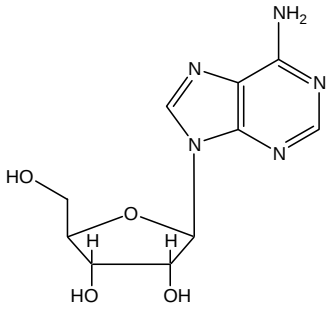
System suitability

System performance: Dissolve 0.1 g of Acyclovir in 5 mL of dilute sodium hydroxide TS, add 2 mL of a solution of guanine in dilute sodium hydroxide TS (1 in 4000), and add the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acyclovir and guanine are eluted in this order with the resolution between these peaks being not less than 17.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acyclovir is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Adenosine



$C_{10}H_{13}N_5O_4$: 267.24

(2*R*,3*R*,4*S*,5*R*)-2-(6-Amino-9*H*-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol [*58-61-7*]

Adenosine contains not less than 99.0 % and not more than 101.0 % of adenosine ($C_{10}H_{13}N_5O_4$), calculated on the dried basis.

Description Adenosine is a colorless crystalline powder and produces adenine and D-ribose by hydrolysis.

Adenosine is freely soluble in water, sparingly soluble in hot water, and slightly soluble in ethanol (95).

Identification Determine the infrared spectra of Adenosine and Adenosine RS, previously dried, as directed in the paste method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 233 ~ 238 ºC

Specific Optical Rotation $[\alpha]_D^{20}$: -68 ~ -72º (after drying, 0.4 g, sodium hydroxide solution (1 in 10), 20 mL, 100 mm).

Purity (1) ***Acidity or alkalinity***—Suspend 1 g of Adenosine in 20 mL carbon dioxide free water. Stir for 30 seconds, and pass through a coarse filter. To each of two 10 mL portions of the filtrate add 0.1 mL of bromocresol purple TS. Not more than 0.3 mL of 0.01 N sodium hydroxide is required to produce a blue-violet color in one portion, and not more than 0.1 mL of 0.01 N hydrochloric acid is required to produce a yellow color in the other portion.

(2) ***Chloride***—Suspend 0.75 g of Adenosine in 15 mL of water. Stir for 30 seconds, pass through a coarse filter, and use the filtrate as the test solution. Prepare a chloride standard solution by diluting 1 mL of sodium chloride solution (231 mg in 1000 mL) with 100 mL of water. To the test solution and 10 mL of the chloride standard solution add 1 mLof nitric acid and 1 mL of silver nitrate TS, dilute each solution with water to 40 mL, and mix. Allow the solutions to stand for 5 minutes, protected from light. When viewed against a dark background, the test solution is not more turbid than the standard solution (not more than 0.007 %).

(3) ***Sulfate***—Suspend 0.75 g of Adenosine in 15 mL of water. Stir for 30 seconds, pass through a coarse filter, and use the filtrate as the test solution. Prepare a sulfate standard solution by adding 0.15 mL of 0.01 mol/L sulfuric acid to 15 mL of water. To the test solution and the standard solution add 2 mL of barium chloride TS and 1 mL of 3 mol/L hydrochloric acid, dilute each solution with water to 30 mL, and mix. Allow the solutions to stand for 5 minutes: the test solution is not more turbid than the standard solution (not more than 0.02 %).

(4) ***Ammonia***— Suspend 0.5 g of Adenosine in 10 mL of water. Stir for 30 seconds, pass through a coarse filter. Dilute the filtrate with water to 15 mL, mix, and use the filtrate as the test solution. Dilute 1 mL of ammonium chloride solution (314 mg in 1000 mL) with 100 mL of water. Mix 2 mL of this ammonia standard solution with 13 mL of water, and use this solution as the reference solution. To the test solution and reference solution add 0.3 mL of alkaline mercuric-

potassium iodide TS, cap the test tubes, and allow to stand for about 5 minutes : the test solution does not exhibit a more intense yellow color than that of the reference solution (not more than 0.0004 %).

(5) ***Heavy metals***—Proceed with 1.0 g of Adenosine according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Related substances***—Weigh accurately 25 mg of Adenosine and dissolve in mobile phase and add to make exactly 25 mL and use this solution as the test solution. Perform the test with exactly with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the percentage of each related substance in the portion of Adenosine taken by the peak area percentage method: not more than 0.2 % of adenine is found; and not more than 0.5 % of total related substances is found.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: A mixture of sulfate buffer and a solution (1 in 10000) of sodium azide (60:40)

Flow rate: 1.5 mL/minute

System suitability

System performance: Weigh accurately each 20 mg of adenosine and 20 mg of inosine and dissolve in mobile phase to make 100 mL volume. When the procedure is run with 20 μL of this solution under the above operating condition, the resolution between adenosine and inosine is not less than 9.0 and the symmetry factor is not more than 2.5.

System repeatability: Weigh 20 mg each of adenosine and inosine, dissolve in mobile phase to make 100 mL. When the test is repeated 5 times with 20 μL each of this solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0 %.

Time span of measurement: Adjust the run time to at least twice the retention time of the major peak.

Sulfate buffer—Dissolve 6.8 g of potassium hydrogen sulfate and 3.4 g of tetrabutylammonium hydrogen sulfate in water, dilute with water to 1000 mL, and mix. Adjust with 2 N potassium hydroxide to a pH of 6.5.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

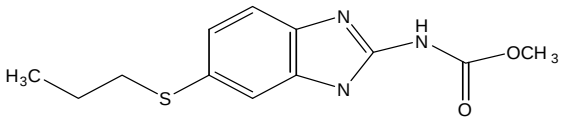
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Adenosine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.724 mg of $C_{10}H_{13}N_5O_4$

Containers and Storage ***Containers***—Tight containers.

Albendazole



$C_{12}H_{15}N_3O_2S$: 265.33

Methyl *N*-(6-propylsulfanyl-1*H*-benzimidazol-2-yl)carbamate [*54965-21-8*]

Albendazole, when dried, contains not less than 98.0 % and not more than 102.0 % of albendazole ($C_{12}H_{15}N_3O_2S$).

Description Albendazole is white to pale yellow powder.
Albendazole is freely soluble in anhydrous formic acid, very slightly soluble in ether or methylene chloride and practically insoluble in water or in ethanol (95).

Identification (1) Perform the test as directed in the Related substances: the principal spots from the test solution and the standard solution show the same R_f value.

(2) Determine the infrared spectra of Albendazole and Albendazole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Related substances***−Dissolve 50 mg of Albendazole in 3 mL of acetic acid (100), add acetic acid (100) to make 5 mL and use this solution as the test solution. Separately, weigh accurately a portion of Albendazole RS, dissolve in the acetic acid (100) to contain 5 mg per mL and use this solution as the standard solution (1). Pipet 1.0 mL of the standard solution, dilute with acetic acid (100) to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with the these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetic acid (100) and ether (60 : 10 : 10) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is

not larger and not more intense than the spot from the standard solution (2). (not more than 0.5 %)

Loss on Drying Not more than 0.5 % (105 ºC, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.25 g of Albendazole, previously dried, dissolve in 100 mL of acetic acid (100), and warm if necessary. After cooling, add 1 drop of solvent blue 19 in acetic acid (100) solution (1 in 200), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes to violet. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.533 mg of $C_{12}H_{15}N_3O_2S$.

Containers and Storage ***Containers***—Well-closed containers.

Albumin Tannate

Tannalubin

Albumin Tannate is a compound of tannic acid and a protein.
The label states the origin of the protein of Albumin Tannate.

Description Albumin Tannate is a pale brown powder. Albumin Tannate is odorless, or has a faint, characteristic odor.
Albumin Tannate is practically insoluble in water or in ethanol (95).
Albumin Tannate dissolves in sodium hydroxide TS with turbidity.

Identification (1) To 0.1 g of Albumin Tannate, add 10 mL of ethanol (95) and heat in a water-bath for 3 minutes with shaking. After cooling, filter and to 5 mL of the filtrate, add 1 drop of iron (III) chloride TS: a blue-purple to bluish black color is produced. On standing, a bluish black precipitate is produced.

(2) To 0.1 g of Albumin Tannate, add 5 mL of nitric acid: an orange-yellow color develops.

Purity (1) ***Acid***—Shake 1.0 g of Albumin Tannate with 50 mL of water for 5 minutes and filter. To 25 mL of the filtrate, add 1.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(2) ***Fats***—To 2.0 g of Albumin Tannate, add 20 mL of petroleum benzine, shake vigorously for 15 minutes and filter. Evaporate 10 mL of the filtrate on a water-bath: the weight of the residue is not more than 50 mg.

Loss on Drying Not more than 6.0 % (1 g, 105 ºC, 3 hours).

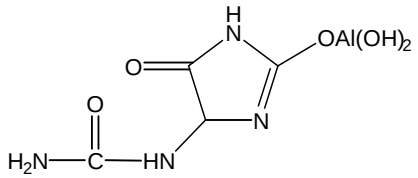
Residue on Ignition Not more than 1.0 % (0.5 g).

Digestion Test To 1.00 g of Albumin Tannate, add 0.25 g of saccharated pepsin and 100 mL of water, shake well and allow to stand for 20 minutes at 40 ± 1 ºC in a water-bath. Add 1.0 mL of dilute hydrochloric acid, shake and allow to stand for 3 hours at 40 ± 1 ºC. Cool rapidly to ordinary temperature and filter. Wash the residue with three 10 mL volumes of water, dry in a desiccator (silica gel) for 18 hours and dry at 105 ºC for 5 hours: the weight of the residue is 0.50 g to 0.58 g.

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant.

Aldioxa



Dihydroxyaluminum Allantoinate

$C_4H_7AlN_4O_5$: 218.10

Aluminium hydroxide 4-(carbamoylamino)-5-oxo-4,5-dihydro-1*H*-imidazol-2-olate (1:2:1) [*5579-81-7*]

Aldioxa is a condensation product of allantoin and aluminum hydroxide. When dried, Aldioxa contains not less than 65.3 % and not more than 74.3 % of allantoin ($C_4H_6N_4O_3$: 158.12) and not less than 11.1 % and not more than 13.0 % of aluminum (Al: 26.98).

Description Aldioxa is a white powder, is odorless and tasteless.
Aldioxa is practically insoluble in water, in ethanol (95) or in ether.
Aldioxa dissolves in dilute hydrochloric acid or in dilute nitric acid.

Melting point—About 230 ºC (with decomposition).

Identification (1) To 0.2 g of Aldioxa, add 10 mL of dilute hydrochloric acid, boil for 5 minutes and add 10 mL of a solution of phenylhydrazine hydrochloride (1 in 100). After cooling, mix well with 0.5 mL of potassium hexacyanoferrate (III) TS and shake with 1 mL of hydrochloric acid: a red color develops.

(2) To 0.2 g of Aldioxa, add 10 mL of dilute hydrochloric acid, dissolve by warming and cool: the solution responds to the Qualitative Tests for aluminum salt.

Purity (1) ***Chloride***—To 0.10 g of Aldioxa, add 6 mL of dilute nitric acid, boil to dissolve with shaking for 5 minutes, cool and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142 %).

(2) ***Nitrate***—To 0.10 g of Aldioxa, add carefully 5 mL of water and 5 mL of sulfuric acid, dissolve by shaking, cool and superimpose 2 mL of iron (II) sulfate TS: no brown ring is produced at the zone of contact.

(3) ***Sulfate***—To 0.20 g of Aldioxa, add 6 mL of dilute hydrochloric acid, boil to dissolve with shaking for 5 minutes, cool and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240 %).

(4) ***Heavy metals***—To 1.0 g of Aldioxa, add 3mL of hydrochloric acid and 3 mL of water, heat gently to boil with shaking and evaporate in a water-bath to dryness. To the residue, add 30 mL of water, shake under warming, cool, filter and to the filtrate, add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3 mL of hydrochloric acid add 3 mL of water, evaporate in a water-bath to dryness and add 2.0 mL of standard lead solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Aldioxa according to Method 2 and perform the test (not more than 2 ppm).

Loss on Drying Not more than 4.0 % (1 g, 105 ºC, 2 hours).

Assay (1) ***Allantoin***—Weigh accurately about 0.1 g of Aldioxa, previously dried, dissolve in 50 mL of dilute sulfuric acid by heating, cool and add water to make exactly 100 mL. Pipet 10 mL of this solution and perform the test as directed under the Nitrogen Determination.

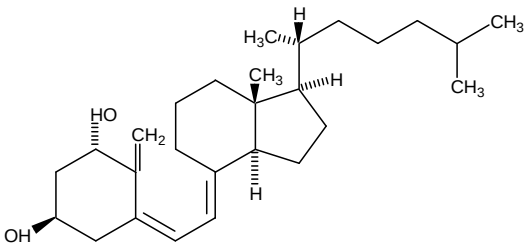
Each mL of 0.005 mol/L sulfuric acid VS
= 0.39529 mg of $C_4H_6N_4O_3$

(2) ***Aluminum***—Weigh accurately about 0.2 g of Aldioxa, previously dried, dissolve carefully in 50 mL of dilute hydrochloric acid by heating, cool and add dilute hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 25 mL and use this solution as the test solution. Separately, pipet a suitable quantity of aluminum standard stock solution, dilute with water so that each mL of the solution contains not less than 16.0 μg and not more than 64.0 μg of aluminum (Al: 26.98) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions and calculate the aluminum content of the test solution from the calibration curve obtained from the absorbance of the standard solution.

Gas: Dissolved acetylene – Nitrous oxide.
Lamp: An aluminum hollow cathode lamp.
Wavelength: 309.2 nm.

Containers and Storage ***Containers***—Well-closed containers.

Alfacalcidol



$C_{27}H_{44}O_2$: 400.64

(1*R*,3*S*,5*Z*)-5-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(2*R*)-6-methylheptan-2-yl]-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol [*41294-56-8*]

Alfacalcidol contains not less than 97.0 % and not more than 102.0 % of alfacalcidol ($C_{27}H_{44}O_2$).

Description Alfacalcidol appears as white crystals.
Alfacalcidol is freely soluble in ethanol (95), soluble in fatty oils, and practically insoluble in water.
Alfacalcidol is sensitive to air, heat and light.
A reversible isomerisation to pre-alfacalcidol takes place in solution, and the activity is due to both compounds.

Identification (1) Determine the infrared spectra of Alfacalcidol and Alfacalcidol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is same in retention time as the principal peak in the chromatogram obtained with standard solution (1).

Purity ***Related substances***—Examine by liquid chromatography as described under Assay. Calculate the percentage content of related substances, other than pre-alfacalcidol, that are eluted within twice the retention time of alfacalcidol from the areas of the peaks in the chromatogram obtained with the test solution by the area percentage method. The content of any individual related substance is not more than 0.5 % and the total area of all the related substances is not more than

1.0 %. Disregard any peak not more than 0.1 %.

Assay Carry out the assay as rapidly as possible, avoiding exposure to light and air. Weigh accurately about 1.0 mg of Alfacalcidol, dissolve in mobile phase to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 1.0 mg of Alfacalcidol RS and dissolve in mobile phase without heating to make exactly 10 mL and use this solution as the standard solution (1). To 1.0 mL of the standard solution (1) add mobile phase to make exactly 100 mL and use this solution as the standard solution (2). Heat 2 ml of the standard solution (1) in a water-bath at 80 ºC under a reflux condenser for 2 hours and cool and use this solution as the standard solution (3). Perform the test with 100 μL each of the test solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area, A_T and A_S, of alfacalcidol by the automatic integration method.

$$\text{Amount (mg) of alfacalcidol } (C_{27}H_{44}O_2) = \text{Amount (mg) of Alfacalcidol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: the mixture of acetonitrile, water and 9 mol/L ammonia water (800 : 200 : 1).

Flow rate: 2.0 mL/minute.

System suitability

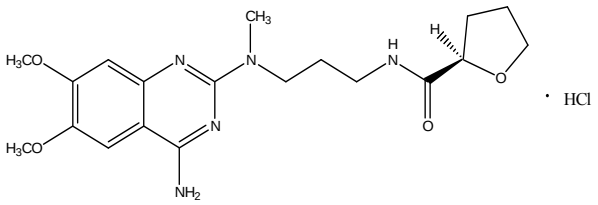
System performance: When the procedure is run with 100 μL of the standard solution (3) under the above operating condition, the retention time for pre-alfacalcidol, relative to alfacalcidol, is about 1.3 with the resolution between the peaks due to pre-alfacalcidol and alfacalcidol being not less than 4.0

System repeatability: When the test is repeated 6 times with 100 μL each of the standard solution (3) under the above operating conditions, the relative standard deviation of the peak areas of alfacalcidol is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant, under nitrogen atmosphere, and at a temperature between 2 ºC and 8 ºC. The contents of an opened container are to be used immediately.

Alfuzosin Hydrochloride



and enantiomer

$C_{19}H_{27}N_5O_4 \cdot HCl$: 425.91

N-{3-[(4-Amino-6,7-dimethoxy-quinazolin-2-yl)methylamino]propyl}tetrahydrofuran- 2-carboxamide hydrochloride [*81403-68-1*]

Alfuzosin Hydrochloride contains not less than 98.5 % and not more than 101.0 % of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$), calculated on the anhydrous basis.

Description Alfuzosin Hydrochloride is a white crystalline powder.

Alfuzosin Hydrochloride is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in dichloromethane.

Alfuzosin Hydrochloride is hygroscopic.

Identification (1) Determine the infrared spectra of Alfuzosin Hydrochloride and Alfuzosin Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) To 0.5 g of Alfuzosin Hydrochloride add 25 mL of water. The diluted solution of 1 mL of this solution by 1 mL of water responds to the Qualitative Tests (2) for chlorides.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10° (0.2 g calculated on the anhydrous basis, water, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.5 g of Alfuzosin Hydrochloride in 25 mL of water containing no carbon dioxide is between 4.0 and 5.5.

Purity ***Related substances***—Weigh accurately 20.0 mg of Alfuzosin Hydrochloride, dissolve in mobile phase to make exactly 100 mL and use this solution as test solution. Pipet 1.0 mL of the test solution, add mobile phase to make exactly 50 mL, pipet 5.0 mL of this solution, add mobile phase to make exactly 20 mL and use this solution as the standard solution (1). Separately, weigh accurately 5 mg of Alfuzosin Related Substance I {*N*-[3-[(4-Amino-6,7-dimethoxyquinolin-2-yl)(methyl)amino] propyl]furan-2-carboxamide} RS, dissolve in mobile phase to make 25 mL, pipet 1 mL of this solution, add 1 mL of the test solution and then add mobile phase to make exactly 100 mL and use this so-

lution as the standard solution (2). Perform the test with exactly 20 μL each of the test and the standard solution (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of any peak other than the principal peak from the test solution is not greater than 0.6 times the area of the principal peak from the standard solution (1) (0.3 %); the total area of all peaks other than the principal peak is not greater than the area of the principal peak with the standard solution (1) (0.5 %). Disregard any peak with an area less than 0.025 times that of the principal peak from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: The mixture of sodium perchlorate solution, acetonitrile and tetrahydrofuran (80 : 20 : 1).

Flow rate: 1.5 mL/minute

System suitability

System performance: Perform the test with 20 μl of standard solution (2) according to the above conditions. Adjust the sensitivity of the system so that the height of the two peaks in the chromatogram obtained is at least 50 % of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to alfuzosin and alfuzosin related substance I is at least 3.0.

Sodium perchlorate solution—Mix 5 mL of perchloric acid and 900 mL of water, adjust pH to 3.5 with 8.5 w/v % sodium hydroxide VS and add water to make 1000 mL.

Water Not more than 0.5 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (1 g).

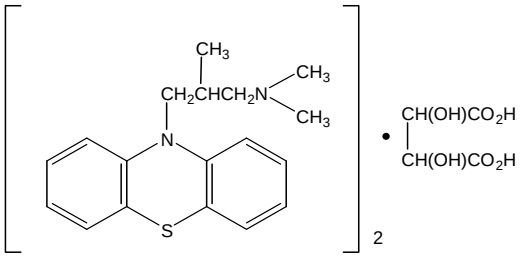
Assay Weigh accurately about 0.3 g of Alfuzosin Hydrochloride, dissolve in the mixture of 40 mL of acetic acid (100) and 40 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.59 mg of $C_{19}H_{28}ClN_5O_4$.

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant.

Alimemazine Tartrate



$(C_{18}H_{22}N_2S)_2{\cdot}C_4H_6O_6$: 746.98

bis-(*N*,*N*,2-Trimethyl-3-phenothiazin-10-yl)propan-1-amine (2*R*,3*R*)-2,3-dihydroxybutanedioate [*4330-99-8*]

Alimemazine Tartrate, when dried, contains not less than 98.0 % and not more than 101.0 % of alimemazine tartrate $(C_{18}H_{22}N_2S)_2{\cdot}\ C_4H_6O_6$).

Description Alimemazine Tartrate is a white powder, is odorless and has a bitter taste.

Alimemazine Tartrate is freely soluble in water or in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in ether.

pH—The pH of a solution of Alimemazine Tartrate (1 in 50) is between 5.0 and 6.5.

Alimemazine Tartrate is gradually colored by light.

Identification (1) To 2 mL of a solution of Alimemazine Tartrate (1 in 100), add 1 drop of iron (III) chloride TS: a red-brown color is produced and immediately a yellow precipitate is formed.

(2) Dissolve 1 g of Alimemazine Tartrate in 5 mL of water, add 3 mL of sodium hydroxide TS, extract with two 10 mL volumes of ether [use the aqueous layer obtained in the Identification (4)]. Shake the combined ether extracts with 3 g of anhydrous sodium sulfate, filter and evaporate the ether with the aid of a current of air. Dry the residue in a desiccator (in vacuum, P_2O_5) for 16 hours: it melts between 66 ºC and 70 ºC.

(3) Determine absorption spectra of solutions of Alimemazine Tartrate and Alimemazine Tartrate RS, in water (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) The aqueous layer, obtained in the Identification (2), when neutralized with dilute acetic acid, responds to the Qualitative Tests (1) and (2) for tartrate.

Melting Point 159 ~ 163 ºC.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Alimemazine Tartrate in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Alimemazine Tartrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL

of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Alimemazine Tartrate according to Method 3 and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 5) (not more than 2 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

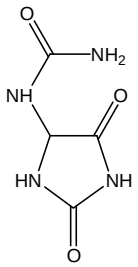
Assay Weigh accurately about 0.8 g of Alimemazine Tartrate, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through brown to green-brown (indicator: 2 mL of 1-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.349 mg of $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant.

Allantoin



$C_4H_6N_4O_3$: 158.12

(2,5-Dioxoimidazolidin-4-yl)urea [*97-59-6*]

Allantoin, when dried, contains not less than 98.5 % and not more than 101.0 % of allantoin ($C_4H_6N_4O_3$).

Description Allantoin is a white crystalline powder.
Allantoin is slightly soluble in water and very slightly soluble in ethanol (95).
Allantoin dissolves in sodium hydroxide TS.

Melting Point— About 225 ºC (decomposition)

Identification (1) Boil 20 mg with a mixture of 1 mL of 2 mol/L sodium hydroxide TS and 1 mL of water. Allow to cool. Add 1 mL of 2 mol/L hydrochloric acid TS. To 0.1 mL of the solution add 0.1 mL of a solution of potassium bromide (1 in 10), 0.1 mL of a solution of resorcinol (1 in 50) and 3 mL of sulfuric acid. Heat for 5 min to 10 min on a water-bath. A dark blue color develops, which becomes red after cooling and pouring into about 10 mL of water.

(2) Determine the infrared spectra of Allantoin and Allantoin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The principal spots in the chromatograms obtained with test solution (2) and the standard solution (1) in Related substances under Purity have the same R_f values.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10º (0.2 g, water, 20 mL, 100 mm)

Purity (1) ***Acidity or alkalinity***—To a 10 mL solution of about 50 mg of Allantoin in carbon dioxide-free water, add 0.1 mL of methyl red TS and 0.2 mL of 0.01 mol/L sodium hydroxide TS: the solution is yellow. Add 0.4 mL of 0.01 mol/L hydrochloric acid TS: the solution is red.

(2) ***Reducing substances***—Mix 1.0 g of Allantoin with 10 mL of water by shaking for 2 minutes and filter. Add 1.5 mL of 0.02 mol/L potassium permanganate TS: the solution must remain purple for at least 10 min.

(3) ***Potassium permangate Related substances***—Weigh accurately about 0.10 g of Allantoin, dissolve in 5 mL of water with heating. After cool add methanol to make exactly 10 mL and use this solution as test solution (1). Pipet 1 mL of the test solution (1), add a mixture of methanol and water (1:1) to make exactly 10 mL and use this solution as the test solution (2). Separately, weigh accurately about 10 mg of Allantoin RS, add a mixture of methanol and water (1 : 1) to make exactly 10 mL and use this solution as the standard solution (1). Weigh accurately about 10 mg of Urea RS, add water to make exactly 10 mL, pipet 1 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution (2). Mix 1 mL each of the standard solution (1) and the standard solution (2) and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL of the test solution (1) and 5 μL each of the test solution (2), the standard solution (1), the standard solution (2) and the standard solution (3) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (60 : 25 : 15) to a distance of about 10 cm and air-dry the plate. Spray evenly the plate with a solution of 0.1 g 4-dimethylaminobenzaldehyde in 20 mL of a mixture of methanol and hydrochloric acid (3 : 1). Dry the plate in a current of hot air. Examine under daylight after 30 min: any spot frorm the test solution (1), other than the principal spot, is not more intense than the spot from standard solution (2) (0.5 %). The test is not valid unless the chromatogram from standard solution (3) shows two clearly separated principal spots.

Loss on Drying Not more than 0.1 % (2 g, 105 °C, constant mass).

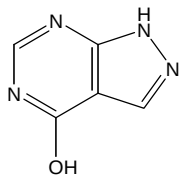
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1.2 g of Allantoin, previously dried, add 40 mL of water to dissolve. Titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.812 mg of $C_4H_6N_4O_3$

Containers and Storage ***Containers***—Well-closed containers.

Allopurinol



$C_5H_4N_4O$: 136.11

1,2-Dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one
[*315-30-0*]

Allopurinol, when dried, contains not less than 98.0 % and not more than 101.0 % of allopurinol ($C_5H_4N_4O$).

Description Allopurinol appears as white to pale yellowish white or crystalline powder.
Allopurinol is slightly soluble in *N,N*-dimethylformamide, and very slightly soluble in water or in ethanol (99.5).
Allopurinol dissolves in ammonia TS.

Identification (1) Dissolve 0.1 g of Allopurinol in 50 mL of water by warming. To 5 mL of this solution, add 1 mL of ammonia TS and 1 mL of silver nitrate TS: a white precipitate is produced.

(2) Determine the absorption spectra of solutions of Allopurinol and Allopurinol RS, in water (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Allopurinol and Allopurinol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Allopurinol in 10 mL of sodium hydroxide TS: the solution is clear and has no more color than Color Matching Fluid D.

(2) ***Sulfate***—To 2.0 g of Allopurinol, add 100 mL of water and boil for 5 minutes. Cool, add water to make 100 mL and filter. To 25 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Allopurinol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Allopurinol according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Hydrazine***—Weigh accurately 0.25 g of Allopurinol, dissolve in 50 mL of a mixture of 2 mol/L sodium hydroxide TS and methanol (1 : 1), add 4 mL of benzaldehyde TS, and allow to stand at room temperature for 2.5 hours. Add 5.0 mL of hexane, shake for 1 minute, allow the layers to separate, and use hexane layer as the test solution. Separately, pipet 5.0 mL each of hydrazine TS and a mixture of 2 mol/L sodium hydroxide TS and methanol (1 : 1), proceed in the same manner as the test solution, and use these solutions as the standard solution and blank solution. Perform the test with 20 μL each of the test solution, standard solution, and blank solution as directed under Liquid Chromatography according to the following conditions: the amount of hydrazine is not more than 10 ppm.

$$\text{Amount (ppm) of hydrazine} = 1000 \times \frac{32.05}{130.12} \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

32.05: Molecular weight of hydrazine
130.12: Molecular weight of hydrazinium sulfate
C_S: Concentration (μg/mL) of hydrazinium sulfate in hydrazine TS
C_T: Concentration (mg/mL) of allopurinol in the test solution
A_T: Peak area of benzalazine obtained from the test solution
A_S: Peak area of benzalazine obtained from the standard solution

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 310 nm)
Column: A stainless steel column about 4 mm in internal diameter and about 25 m in length, packed with nitrile silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 30 °C
Mobile phase: A mixture of hexane and 2-propanol (95 : 5)
Flow rate: 1.5 mL/minute

System suitability

System performance: When the procedure is run with 20 μL each of the standard solution under the above operating conditions, the relative retention time of benzalazine with respect to benzaldehyde is 0.8 with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of benzalazine is not more than 15.0 %.

(6) ***Related substances***—Dissolve 50 mg of Allopurinol in 10 mL of ammonia TS and use this solution as the test solution. Pipet 1 mL of this solution, add ammonia TS to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with ammonia TS saturated with 1-butanol to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.16 g of Allopurinol, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide by warming. Cool and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Separately to 70 mL of dimethylformamide, add 12 mL of water, perform a blank determination with this solution and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.611 mg of $C_5H_4N_4O$

Containers and Storage ***Containers***—Tight containers.

Allopurinol Tablets

Allpurinol Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of allopurinol ($C_5H_4N_4O$: 136.11).

Method of Preparation Prepare as directed under Tablets, with Allopurinol.

Identification (1) Determine the absorption spectrum of the test solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 248 nm and 252 nm.

(2) To an amount of powdered Allopurinol Tablets, equivalent to 50 mg of Allopurinol according to the labeled amount, add 10 mL of 0.1 mol/L sodium hydroxide VS, mix thoroughly and extract. After filtration, make the filtrate acidic with 1 mol/L acetic acid. Collect the precipitate, wash few times with 3 mL of ethanol (99.5), 4 mL of anhydrous ether, dry in air for 15 minutes and dry at 105 °C for 3 hours. Determine the infrared spectra of the residue and Allopurinol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) To an amount of powdered Allopurinol Tablets, equivalent to 0.1 g of Allopurinol according to the labeled amount, add 5 mL of a solution of diethylamine (1 in 10), and shake well. To this solution add 5 mL of methanol, centrifuge, and use the clear supernatant liquid as the test solution. Separately, to 0.1 g of Allopurinol RS add 5 mL of a solution of diethylamine (1 in 10) and shake well. To this solution add 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2.5 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, ammonia solution (28), and 2-methoxyethanol (3 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the test solution and standard solution have the same R_f value.

Dissolution Test Perform the test with 1 tablet of Allopurinol Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of 0.1 mol/L hydrochloric acid as the dissolution solution. Take a volume of the dissolved solution 45 minutes after starting the test and filter through a membrane filter, dilute with dissolution solution and use this solution as the test solution. Separately, weigh accurately a portion of Allopurinol RS, dissolve in the dissolution solution to make the same concentration with the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at approximately 250 nm as directed under the Ultrviolet-visible Spectrophotometry.
The dissolution rate of Allopurinol Tablets in 45 minutes should be not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 tablets of Allopurinol Tablets. Weigh accurately a por-

tion of the powder, equivalent to about 50 mg of allopurinol ($C_5H_4N_4O$), add exactly 10 mL of 0.1 mol/L sodium hydroxide VS, shake for 10 minutes and add water to make exactly 50 mL (perform the following assay as soon as possible). After centrifugation, discard 10 mL of the first filtrate, collect exactly 4 mL of the subsequent filtrate, add exactly 2.0 mL of the internal standard solution and the mobile phase to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Allopurinol RS, previously dried at 105 °C in vacuum for 5 hours, dissolve in 10 mL of 0.1 mol/L sodium hydroxide VS, mix in shaker for 10 minutes and add water to make exactly 50 mL. To exactly 4 mL of this solution, add exactly 2 mL of the internal standard solution and the mobile phase to make 200 mL and use this solution as the standard solution (prepare freshly when used). Perform the test with 15 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Allopurinol to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of allopurinol } (C_5H_4N_4O) = \text{Amount (mg) of Allopurinol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 50 mg of hypoxantin and 10 mL of 0.1 mol/L sodium hydroxide VS, mix in shaker, add water to make exactly 50 mL. Prepare freshly when used.

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: 0.05 mol/L dibasic ammonium phosphate solution.

Flow rate: 1.5 mL/minute.

System suitability

System performance: When procedure is run with 15 μL of the standard solution under the above operating conditions. Hypoxanthin and allopurinol are eluted in this order with the resolution between their peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 15 μL each of the standard solution, the relative standard deviation of the ratios of the peak area of alloprinol to that of internal standard is not more than 3.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Almagate

$Al_2Mg_6C_2H_{14}O_{20}\cdot 4H_2O$: 630

Aluminum trimagnesium carbonate heptahydroxide dihydrate [*66827-12-1*]

Almagate contains not less than 15.0 % and not more than 17.0 % of aluminum oxide (Al_2O_3: 101.96), not less than 36.0 % and not more than 40.0 % of magnesium oxide (MgO: 40.30), and not less than 12.5 % and not more than 14.5 % of carbon dioxide (CO_2: 44.01).

Description Almagate appears as white, fine crystalline powder.

Almagate is practically insoluble in water, in ethanol (95), or in dichloromethane.

Almagate dissolves with effervescence and heating in dilute mineral acids.

Identification (1) Determine the infrared spectra of Almagate and Almagate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 0.15 g of Almagate in dilute hydrochloric acid to make 20 mL. To 2 mL of this solution, add about 0.5 mL of dilute hydrochloric acid and about 0.5 mL of thioacetamide TS: no precipitate is produced. To this solution, drop 2 mol/L sodium hydroxide TS: a white gelatin-like precipitate is produced. To this solution, add more 2 mol/L sodium hydroxide TS: the precipitate dissolves. To this solution, gradually add more ammonium chloride TS: a white gelatin-like precipitate is produced again.

(3) Dissolve 0.15 g of Almagate in dilute hydrochloric acid to make 20 mL. To 2 mL of this solution, add 1 mL of ammonia TS : a white precipitate is produced. To this solution, add 1 mL of ammoniun chloride TS: the precipitate dissolves. To this solution, add 1 mL of disodium hydrogen phosphate dodecahydrate solution (9 in 100): a white crystalline precipitate is produced.

pH Disperse 4.0 g of Almagate in 100 mL of carbon dioxide-free water, mix for 2 minutes and filter: the pH of this solution is between 8.4 and 10.4.

Purity (1) ***Chloride***—Dissolve 0.33 g of Almagate in 5 mL of dilute nitric acid, add water to make 100 mL, and use this solution as the test solution. Prepare the control solution by adding 0.7 mL of dilute nitric acid and 5 mL of water to 10 mL of chloride standard solution. To 15 mL each of the test solution and control solution, add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS, allow to stand for 5 minutes protected from direct sunlight, and compare the turbidity of these solutions in Nessler tubes against a black background: the turbidity of the test solution is not more intense than that of the control solution (not more than 0.1 %).

Chloride standard solution—Weigh accurately 0.824 g of sodium chloride, and add water to make exactly 1000 mL. To 1.0 mL of this solution, add water to make exactly 1000 mL before use.

(2) ***Sulfate***—Dissolve 0.25 g of Almagate in 5 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the test solution. Prepare the control solution by adding 0.8 mL of dilute hydrochloric acid to 15 mL of sulfate standard solution. Allow a mixture of 3 mL of barium chlorid TS and 4.5 mL of sulfate standard solution to stand for 1 minute. Add 2.5 mL each of this solution to 15 mL each of the test solution and control solution, add 0.5 mL of acetic acid (31), allow to stand for 5 minutes, and compare the turbidity of these solutions: the turbidity of the test solution is not more intense than that of the control solution (not more than 0.4 %).

Sulfate standard solution—Dissolve 0.181 g of potassium sulfate in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL. Prepare this solution immediately before use.

(3) ***Sodium***—Dissolve 0.25 g of Almagate in 50 mL of 3 mol/L hydrochloric acid, and use this solution as the test solution. Separately, weigh accurately 3.050 g of Sodium Chloride RS, previously dried at 130 ℃ for 2 hours, dissolve in water to make exactly 1000 mL so that each mL contains 1.20 mg of sodium, and use this solution as the standard solution. If necessary, dilute with 3 mol/L hydrochloric acid TS. Perform the test with the test solution and standard solution according to Atomic Absorption Spectrophotometry under the following conditions, and determine the amount of sodium in the test solution, using the calibration curve obtained from the standard solution (not more than 1000 ppm).

Gas used : Dissolved acetylene−Air.
Lamp: A sodium hollow cathode lamp.
Wavelength: 589.0 nm.

(4) ***Heavy metals***—Dissolve 1.0 g of Almagate in dilute hydrochloric acid to make 20 mL. Take 12 mL of this solution, use this solution as the test solution. Prepare the control solution by adding 2 mL of the test solution to 10 mL of diluted standard lead solution. Prepare the blank solution by adding 2 mL of the test solution solution to 10 mL of water. To 12 mL each of the test solution, control solution and blank solution, add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. After allow to stand for 2 minutes, the brown color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

Diluted standard lead solution—Pipet 5 mL of standard led solution, add water to make 50 mL. Prepare this solution immediately before use.

System suitability: The control solution has a slightly more brown color than the blank solution.

Loss on Ignition 43.0 ~ 49.0 % (1 g, 900 ± 50 ℃).

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g and *Escherichia coli* and *Pseudomonas aeruginosa* are not observed.

Acid-Consuming Capacity Weigh accurately 0.5 g of Almagate, disperse in 100 mL of water, warm at 37 ± 2 ℃, add 100 mL of 0.1 mol/L hydrochloric acid, previously warmed at 37 ± 2 ℃, and stir continuously: the pH of this solution is maintained between 3.0 and 4.5 for 5 to 20 minutes. Add 10 mL of 0.5 mol/L hydrochloric acid, previously warmed at 37 ± 2 ℃, stir for 1 hour in a water bath of 37 ± 2 ℃, and titrate with 0.1 mol/L sodium hydroxide VS until the pH of this solution becomes to pH 3.5: not more than 20 mL is consumed.

Assay (1) ***Aluminium***—Weigh accurately about 1.0 g of Almagate, dissolve in 5 mL of hydrochloric acid, and heat if necessary. Allow this solution to cool to room temperature, and add water to make exactly 100 mL. Pipet 10 mL of this solution, transfer into a 250 mL conical flask, and add 25 mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS, 20 mL of pH 3.5 acetate buffer solution, 40 mL of ethanol (95) and 2 mL of freshly prepared dithizone TS. Titrate the excess disodium ethylenediaminetetraacetate with 0.05 mol/L zinc sulfate until the color changes from green-purple to pink.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.549 mg of Al_2O_3

(2) ***Magnesium***—Weigh accurately about 1.0 g of Almagate, dissolve in 5 mL of hydrochloric acid, and heat if necessary. Allow this solution to cool to room temperature, and add water to make exactly 100 mL. Pipet 10 mL of this solution, transfer into a 250 mL conical flask, add 200 mL of water and 20 mL of 2,2',2"−nitriletriethanol (95) while shaking, and add 10 mL of pH 10.0 ammonium chloride buffer solution and 50 mg of eriochrome black T-sodium chloride indicator. Titrate this solution with 0.05 mol/L disodium ethylenediamine tetraacetate VS until the color changes from violet to blue.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.015 mg of MgO

(2) ***Carbonic acid***—Weigh accurately about 200 mg of Almagate, transfer into the flask (B), and add 50 mL of water. Add 2 to 3 drops of methyl orange TS in the flask (B). Put boiling chips, connect the funnel (A) and the flask (B), and close the control valve (F). Separately, put 25 mL of 0.1 mol/L sodium hydroxide TS into the flask (J), add immediately 300 mL of barium chloride solution (1 in 100), stir, and connect the flask (J) and the flask (H). Connect the control valve (F) and the flask (H) using the rubber tube, and open the control valve (F). Perform this procedure quickly. Put 50 mL of 1 mol/L hydrochloric acid TS into the funnel (A), open the round valve (L), heat the flask (B) until to boiling, and heat for 10 more minutes. Immediately after heating, close the control valve (F) and the round valve (L), and disconnect the rubber tube (D_2) and the glass tube (E). Shake vigorously the disconnected flask (J) and (H) for 2 minutes, allow to stand for 10 minutes at a room temperature, and open the control valve (F) and the round valve (L). Filtrate the solution in the flask (J) under reduced pressure, add 2 to 3 drops of methyl orange TS, and titrate with 0.1 mol/L hydrochloric acid until the color changes from pale orange to red. Perform a blank determination and make any necessary correction.

$$\text{Amount (\%) of carbonic acid} = \frac{(a-b)\times f}{\text{amount (mg) of the sample taken}} \times 2.200 \times 100$$

Each mL of 0.1 mol/L hydrochloric acid TS
= 2.200 mg of CO_2

a: Volume (mL) of 0.1 mol/L hydrochloric acid TS consumed in the blank test

b: Volume (mL) of 0.1 mol/L hydrochloric acid TS consumed in the test

f: Normality factor of 0.1 mol/L hydrochloric acid

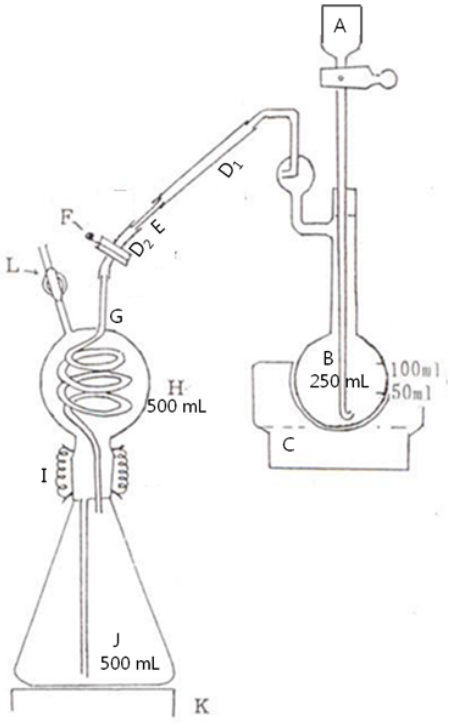
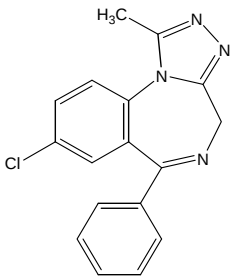


Fig. Carbon dioxide measuring apparatus

A: Funnel for adding the acid to decompose the sample
B: Flask for decomposing the sample
C: Mantle
D_1, D_2: Rubber tube
E: Glass tube
F: Control valve
G: Cooling glass tube
H: Safety maintenance flask
I: Spring vessel
J: CO_2 absorption flask
K: Stirring apparatus
L: Round valve

Containers and Storage ***Containers***—Tight containers.

Alprazolam



$C_{17}H_{13}ClN_4$: 308.77

8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine [*28981-97-7*]

Alprazolam, when dried, contains not less than 98.5 % and not more than 101.0 % of alprazolam ($C_{17}H_{13}ClN_4$).

Description Alprazolam appears as white crystals or crystalline powder.
Alprazolam is freely soluble in chloroform, soluble in methanol or in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in water.
Alprazolam dissolves in dilute nitric acid.

Identification (1) Determine the absorption spectrum of a solution of Alprazolam in ethanol (95) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maximum at around 222 nm.

(2) Dissolve 50 mg of Alprazolam in 0.7 mL of deuterochloroform for nuclear magnetic resonance spectroscopy, and determine the ^1H spectrum of this using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits singlet signal A at around δ 2.6 ppm, doublet signals B and C at around δ 4.0 ppm and δ 5.4 ppm, and a broad signal D between δ 7.1 ppm and δ 7.96 ppm. The ratio of integrated intensity of each signal, A : B : C : D, is about 3 : 1 : 1 : 8.

(3) Perform the test with Alprazolam as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 228 ~ 232 ºC.

Purity (1) ***Chloride***—Dissolve 0.5 g of Alprazolam in 10 mL of dilute nitric acid, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Alprazolam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50 mg of Alprazolam in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, then pipet 1 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plates with an upper layer of a mixture of acetone, hexane, ethyl acetate and ethanol (95) (4 : 2 : 2 : 1) to a distance of about 10 cm, and air-dry the plates. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum at the pressure not exceeding 0.67 kPa, 60 ºC, 4 hours).

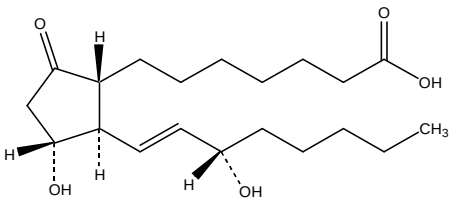
Residue on Ignition Not more than 0.1 % (1.0 g).

Assay Weigh accurately about 0.25 g of Alprazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.438 mg of $C_{17}H_{13}ClN_4$

Containers and Storage ***Containers***—Tight containers.

Alprostadil



Prostaglandin E_1 $C_{20}H_{34}O_5$: 354.48

7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-1-yl}heptanoic acid [*745-65-3*]

Alprostadil, when dried, contains not less than 97.0 % and not more than 103.0 % of alprostadil ($C_{20}H_{34}O_5$).

Description Alprostadil appears as white crystals or crystalline powder.
Alprostadil is freely soluble in ethanol (99.5) or tetrahydrofuran, slightly soluble in acetonitrile, and practically insoluble in water.

Identification (1) To 10 mL each of the solutions of Alprostadil and Alprostadil RS, respectively, in ethanol (99.5) (1 in 100000) add 1 mL of potassium hydroxide·ethanol TS and allow to stand for 15 minutes. Determine the absorption spectra of the solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Alprostadil and Alprostadil RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -53 ~ -61º (after

drying, 25 mg, tetrahydrofuran, 5 mL, 100 mm)

Melting Point 114 ~ 118 ºC.

Purity ***Related substances***—Dissolve 4 mg of Alprostadil in 2 mL of a mixture of acetonitrile and water (9 : 1) and use this solution as the test solution. Pipet 0.5 mL of this solution, add a mixture of acetonitrile and water (9 : 1) to make exactly 10 mL, pipet 2.0 mL of this solution, add a mixture of acetonitrile and water (9 : 1) to make exactly 10 mL and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks being about 0.70 and about 1.26 in relative retention time reference to the peak of alprostadil in the test solution are not larger than 0.5 times the peak area of alprostadil obtained with the standard solution; the areas of the peaks being about 0.88 and about 1.18 in relative retention time reference to alprostadil in test solution are not larger than the peak area of alprostadil obtained with the standard solution; the area of the peaks other than alprostadil and above mentioned peaks in the test solution is not larger than 0.1 times the peak area of alprostadil in standard solution. The total area of all peaks other than alprostadil is not larger than 2 times the peak area of alprostadil from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate: Perform as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, add a mixture of acetonitrile and water (9 : 1) to make 20 mL. Confirm that the peak area of alprostadil obtained with 5 μL of this solution is equivalent to 7 to 13 % of that with 5 μL of the standard solution.

System performance: Perform as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alprostadil is not more than 1.5 %.

Time span of measurement: About 5 times as long as the retention time of alprostadil beginning after the solvent peak.

Loss on Drying Not more than 1.0 % (0.1 g, in vacuum, P_2O_5, 4 hours).

Assay Weigh accurately about 5 mg each of Alprostadil and Alprostadil RS, previously dried, to each add exactly 5 mL of internal standard solution to dissolve, add a mixture of acetonitrile and water (9 : 1) to make exactly 50 mL, and use the solutions as test solution and standard solution, respectively. Perform the test with 5 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of alprostadil to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of alprostadil } (C_{20}H_{34}O_5) = \text{Amount (mg) of Alprostadil RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dimethyl phthalate in a mixture of acetonitrile and water (9 : 1) (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 196 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL, add the solution prepared by dissolving 9.46 g of potassium monohydrogen phosphate in water to make 1000 mL to adjust pH to 6.3. Dilute this solution 10 times with water. To 360 mL of this solution add 110 mL of acetonitrile and 30 mL of methanol and mix.

Flow rate: Adjust the flow rate so that the retention time of alprostadil is about 10 minutes.

System suitability

System performance: When the prodedure is run with 5 μL of the standard solution, as diredcted under the above operating, alprostadil and the internal standard are eluted in this order with the resolution between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution, as directed under the above conditions, relative standard deviation of the ratios of the peak area of alprostadil reference to the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant, and not exceeding 5 ºC.

Aluminum Hydroxide Gel

$AI(OH)_3$: 78.00

Aluminum Hydroxide Gel is a suspension of aluminum hydroxide. Aluminum Hydroxide Gel contains not less

than 3.6 % and not more than 4.4 % of the labeled amount of aluminum oxide (Al_2O_3: 101.96) when analyzed as aluminum hydroxide suspension. Aluminum Hydroxide Gel may contain peppermint oil, glycerin, sorbitol, sucrose, saccharin, or other suitable flavors and suitable antimicrobial agents.

Description Aluminum Hydroxide Gel is a white, viscous suspension. A volume of water layer is separated on standing.

Identification Dissolve approximately 5 mL of Aluminum Hydroxide Gel in 10 mL of dilute hydrochloride acid and warm: the solution responds to Qualitative Tests for aluminum salt.

pH 5.5 ~ 8.0.

Purity (1) ***Chloride***—Transfer an accurately measured volume of Aluminum Hydroxide Gel, equivalent to 10 g of $Al(OH)_3$, to a porcelain dish. Add 0.1 mL of potassium chromate TS and 25 mL of water. Stir and add 0.10 mol/L of silver nitrate until a pale red color is obtained: not more than 8.0 mL of 0.10 mol/L nitrate is required (not more than 0.28 %).

(2) ***Sulfate***—Add 5.0 mL of 3 mol/L of hydrochloric acid to an accurately measured volume of Aluminum Hydroxide Gel, equivalent to 5 g of Aluminum Hydroxide Gel and heat to dissolve. Cool, dilute with water to make 250 mL and filter, if necessary. Add 1 mL of dilute hydrochloric acid to a 20 mL portion of the filtrate, dilute with water to make 50 mL and use this solution as the test solution. 0.40 mL of 0.005 mol/L sulfuric acid are added to reference standard (not more than 0.05 %).

(3) ***Arsenic***—Add 10 mL of dilute sulfuric acid to 1.2 g of Aluminum Hydroxide Gel, shake, heat to boiling and cool. Dilute with water to make 20 mL and filter. Use 10 mL of the filtrate as the test solution. Perform the test (not more than 0.6 ppm).

(4) ***Heavy metal***—Add 5 mL of dilute hydrochloric acid to 5.0 g of Aluminum Hydroxide Gel, shake, heat to boiling and evaporate in a water-bath to dryness. Add 30 mL of water to the residue, warm, shake, cool and filter. Add 2 mL of dilute acetic acid and dilute with water to make 50 mL. Use this solution as the test solution and perform the test. Evaporate 5 mL of dilute hydrochloric acid in a water-bath. Add 2.5 mL of the standard lead solution and 2 mL of diluted acetic acid. Dilute with water to make 50 mL and use this solution as the control solution (not more than 5 ppm).

Acid-Consuming Capacity Add about 1.5 mL of Aluminum Hydroxide Gel in a flask equipped with a stopper, previously weighed. Weigh accurately and perform the test: The volume of 0.1 mol/L of hydrochloric acid VS consumed is 12.5 to 25.0 mL per g of Aluminum Hydroxide Gel.

Microbial Limit The total anaerobic microbial count is not more than 100 CFU/mL and *Escherichia coli* is not observed.

Assay Transfer an accurately measured volume of Aluminum Hydroxide Gel, equivalent to about 25 g of Aluminum Hydroxide Gel, to a beaker, add 15 mL of hydrochloric acid and heat gently until solution is clear. Cool, transfer to a volumetric flask, dilute with water to make 500 mL and mix. Pipet 20 mL of this solution into a beaker and add 25.0 mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS and 20 mL of acetic acid· ammonium acetate buffer TS (pH 4.8), with continuous stirring then heat near the boiling point for 5 minutes. Cool and add 50 mL of ethanol and 2 mL of dithizone TS. Titrate the solution with 0.05 mol/L of zinc sulfate VS until the color changes from green-violet to red. Perform a blank determination, substituting 20 mL of water for the sample and make any necessary correction.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.5490 mg of Al_2O_3

Containers and Storage ***Containers***—Tight containers.

Storage—Avoid freezing.

Dried Aluminum Hydroxide Gel

Dried Aluminum Hydroxide gel contains not less than 50.0 % of aluminum oxide (Al_2O_3: 101.96).

Description Dried Aluminum Hydroxide Gel is a white and amorphous powder. Dried Aluminum Hydroxide gel is odorless and has no taste.
Dried Aluminum Hydroxide Gel is practically insoluble in water, in ethanol (95) or in ether.
Most of Dried Aluminum Hydroxide Gel is soluble in dilute hydrochloric acid or in sodium hydroxide TS.

Identification Dissolve 0.2 g of Dried Aluminum Hydroxide Gel in 20 mL of dilute hydrochloric acid by warming and centrifuge. The clear supernatant liquid responds to Qualitative test for aluminum salt.

Purity (1) ***Acidity of solution***—Add 1.0 g of Dried Aluminum Hydroxide Gel in 25 mL of water and centrifuge: the clear supernatant liquid is neutral.

(2) ***Chloride***—Add 1.0 g of Dried Aluminum Hydroxide Gel to 30 mL of dilute hydrochloric acid, shake and heat gently to boiling. Cool, dilute with water to make 100 mL and centrifuge. Add 6 mL of dilute hydronitric acid to 5 mL of the clear supernatant liquid and dilute with water to make 50 mL. Use this solution as the test solution. Prepare the control solution with 0.4 mL of 0.01 mol/L of hydrochloric acid (not more than 0.284 %).

(3) ***Nitrate***—Add 0.1 g of Dried Aluminum Hydroxide Gel to 5 mL of water. Add 5 mL of sulfuric acid carefully to dissolve, shake and cool. No brown ring is produced when ferric chloride is added dropwise.

(4) ***Sulfate***—Add 1.0 g of Dried Aluminum Hydroxide Gel to 15 mL of dilute hydrochloric acid. shake and heat gently to boiling. Cool, dilute with water to make 250 mL and centrifuge. Add 1 mL of dilute hydrochloric acid to 25 mL of the clear supernatant liquid and dilute with water to make 50 mL. Use this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L of hydrochloric acid (not more than 0.480 %).

(5) ***Heavy metal***—Add 2 g of Dried Aluminum Hydroxide Gel to 10 mL of dilute hydrochloric acid and dilute with water to make 50 mL. Use this solution as the test solution and perform the test. Evaporate 10 mL of dilute hydrochloric acid to dryness. Add 2.0 mL of the standard lead solution and 2 mL of dilute acetic acid. Dilute with water to make 50 mL (not more than 10 ppm).

(6) ***Arsenic***—To 0.8 g of Dried Aluminum Hydroxide Gel, add 10 mL of dilute sulfuric acid, heat gently to boil while shaking, cooling and filter. Take 5 mL of the filtrate, use this solution as the test solution and perform the test (not more than 5 ppm).

Acid-Consuming Capacity Weigh precisely about 0.2 g of Dried Aluminum Hydroxide Gel in a flask equipped with a stopper and perform the test: The volume of 0.1 mol/L of hydrochloric acid VS consumed is not less than 250 mL per 1 g of Aluminum Hydroxide Gel.

Assay Transfer an accurately measured quantity of Dried Aluminum Hydroxide Gel, equivalent to about 2 g of Aluminum Hydroxide Gel, to a beaker, add 15 mL of hydrochloric acid and heat gently until solution is clear. Cool, transfer to a volumetric flask, dilute with water to make 500 mL and mix. Pipet 20 mL of this solution into a beaker and add 30 mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS and 20 mL of acetic acid ammonium acetate buffer TS (pH 4.8) with continuous stirring, then heat the solution near the boiling point for 5 minutes. Cool and add 55 mL of ethanol (95) and 2 mL of dithizone TS. Titrate the solution with 0.05 mol/L of zinc acetate VS until the color changes from pale dark green to pale red. Perform a blank determination, substituting 20 mL of water for the sample and make any necessary correction.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.5490 mg of Al_2O_3

Containers and Storage ***Containers***— Tight containers.

Dried Aluminum Hydroxide Gel Fine Granules

Dried Aluminum Hydroxide Gel Fine Granules contain not less than 47.0 % of aluminum oxide (Al_2O_3: 101.96).

Method of Preparation Prepare as directed under Granules, with Dried Aluminum Hydroxide Gel.

Identification Dissolve 0.2 g of Dried Aluminum Hydroxide Gel in 20 mL of dilute hydrochloric acid by warming and centrifuge. The clear supernatant liquid responds to Qualitative Test for aluminum salt.

Particle Size Distribution Test It meets the requirement.

Acid-Consuming Capacity Proceed as directed in the Acid-neutralizing Capacity under Dried Aluminum Hydroxide Gel: the volume of 0.1 mol/L of hydrochloric acid VS consumed is not less than 235 mL per 1 g of Aluminum Hydroxide Gel.

Assay Proceed as directed in the Assay under Dried Aluminum Hydroxide Gel.

Each mL of 0.05 mol/L of disodium
ethylenediaminetetraacetate VS = 2.5490 mg of Al_2O_3

Containers and Storage ***Containers***— Tight containers.

Natural Aluminum Silicate

Description Natural Aluminum Silicate is a white or slightly colored powder, is odorless and tasteless.
Natural Aluminum Silicate is practically insoluble in water, in ethanol (95) or in ether.
Natural Aluminum Silicate, 1 g, dissolves when heated in 20 mL of sodium hydroxide solution (1 in 5), with some decomposition, leaving a large amount of insoluble substance.

Identification (1) To 0.5 g of Natural Aluminum Silicate, add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water and filter. Render the filtrate slightly acidic with ammonia TS: the solution responds to the Qualitative Tests for aluminum salt.

(2) Prepare a bead by fusing dibasic sodium ammonium phosphate tetrahydrate on a platinum loop. Place the bead in contact with Natural Aluminum Silicate and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

Purity (1) ***Acidity or alkalinity***—Shake 5.0 g of Natural Aluminum Silicate with 100 mL of water and centrifuge: the clear supernatant liquid so obtained is neutral.

(2) ***Chloride***—To 5.0 g of Natural Aluminum Silicate, add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume and centrifuge. To 10 mL of the clear supernatant liquid, add 6 mL of dilute nitric acid, dilute to 50 mL with water and perform the test with this solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

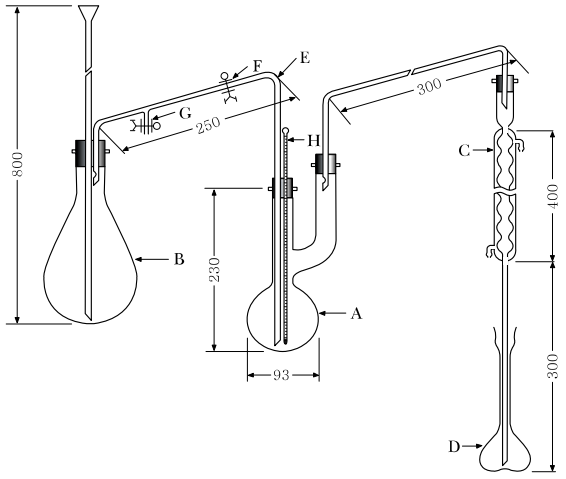
(3) ***Sulfate***—To the residue obtained in (6), add 3 mL of dilute hydrochloric acid, heat on a water-bath for 10 minutes, dilute with 50 mL of water and filter. To 2.0 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480 %).

(4) ***Heavy metals***—To 1.5 g of Natural Aluminum Silicate, add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then cool, centrifuge, remove the clear supernatant liquid, wash the residue with two 10 mL volumes of water, centrifuging each time, combine these washings with the filtrate and add ammonia solution (28) drop-wise until a precipitate just produces. Add dilute hydrochloric acid drop-wise with vigorous shaking and redissolve the precipitate. Heat the mixture with 0.45 g of hydroxylamine hydrochloride, cool and add 0.45 g of sodium acetate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution, 0.15 g of hydroxylamine hydrochloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(5) ***Arsenic***—To 1.0 g of Natural Aluminum Silicate, add 5 mL of dilute hydrochloric acid, heat gently to boil while shaking well, cool rapidly and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue and repeat the extraction in the same manner. Concentrate the combined extracts in a water-bath to 5 mL, use this solution as the test solution and perform the test (not more than 2 ppm).

(6) ***Soluble salts***—Evaporate 50 mL of the clear supernatant liquid obtained in (1) on a water-bath to dryness and ignite the residue at 700 ºC for 2 hours: the weight of the ignited residue is not more than 40 mg.

(7) ***Fluoride***—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.



The figures are in mm.

A: Distilling flask of about 300-mL capacity

B: Steam generator of about 1000-mL capacity, add a boiling stone to prevent bumping

C: Condenser

D: Receiver (200-mL volumetric flask)

E: Steam-introducing tube having an internal diameter of about 8 mm

F, G: Rubber tube with a pinch-coke

H: Thermometer

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass fiber and 50 mL of diluted purified sulfuric acid (1 in 2) and connect A to the distillation apparatus, previously washed with steam through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130 ºC, then open the rubber tube F, close the rubber tube G and boil water in the steam generator B vigorously. Simultaneously, heat A and maintain the temperature of the solution in A between 135 ºC and 145 ºC Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under the Oxygen Flask Combustion Method and determine the amount of fluoride (F) in the test solution according to the following equation: not more than 0.01 %. No corrective solution is used in this procedure.

$$\text{Amount (mg) of fluoride (F: 19.00) in the test solution} = \text{Amount (mg) of fluoride in 5 mL of the standard solution} \times \frac{A_T}{A_S} \times \frac{200}{V}$$

Loss on Drying Not more than 20.0 % (1 g, 105 ºC, 3 hours).

Adsorptive Power To 0.10 g of Natural Aluminum Silicate, add 20 mL of a solution of methylene blue (3 in 2000), shake for 15 minutes, allow to stand for 5 hours at 37 ± 2 ºC and centrifuge. Dilute 1.0 mL of the clear supernatant liquid with water to make 200 mL. Place 50 mL of the solution in a Nessler tube and observe horizontally or vertically against a white background: the color of the solution is not more intense than that of the following control solution.

Control solution—Dilute 1.0 mL of a solution of methylene blue (3 in 2000) with water to make 400 mL and use 50 mL of this solution.

Containers and Storage ***Containers***—Well-closed containers.

Synthetic Aluminum Silicate

Description Synthetic Aluminum Silicate is a white powder, is odorless and tasteless.
Synthetic Aluminum Silicate is practically insoluble in water, in ethanol (95) or in ether.
Synthetic Aluminum Silicate, 1 g, dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), leaving a small amount of insoluble substance.

Identification Proceed as directed in the Identification under Natural Aluminum Silicate.

Purity (1) ***Acidity or alkalinity***—Shake 1.0 g of Synthetic Aluminum Silicate with 20 mL of water and centrifuge: the clear supernatant liquid so obtained is neutral.

(2) ***Chloride***—Proceed as directed in the Purity under Natural Aluminum Silicate.

(3) ***Sulfate***—To 2.0 mL of the clear supernatant liquid obtained in (2), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480 %).

(4) ***Heavy metals***—Weigh about 3.0 g of Synthetic Aluminum Silicate and proceed as directed in the Purity (4) under Natural Aluminum Silicate. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

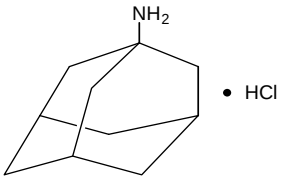
(5) ***Arsenic***—To 1.0 g of Synthetic Aluminum Silicate, add 10 mL of dilute hydrochloric acid, proceed as directed in the Purity (5) under Natural Aluminum Silicate (not more than 2 ppm).

Loss on Drying Not more than 20.0 % (1 g, 105 ºC, 3 hours).

Test for Acid-Neutralizing Capacity Weigh accurately about 1g of Synthetic Aluminum Silicate, transfer to a glass-stoppered flask, add 200 mL of 0.1 mol/L hydrochloric acid VS, exactly measured, stopper the flask, and shake at 37 ± 2 ºC for 1 hour. Filter, pipet 50 mL of the filtrate, and titrate by stirring well the excess hydrochloric acid with 0.1mol/L sodium hydroxide VS until the pH of the solution changes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 50.0 mL per g of Synthetic Aluminum Silicate.

Containers and Storage ***Containers***—Well-closed containers.

Amantadine Hydrochloride



$C_{10}H_{17}N \cdot HCl$: 187.71

Adamantan-1-amine hydrochloride [*665-66-7*]

Amantadine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$).

Description Amantadine Hydrochloride is a white crystalline powder, is odorless and has a bitter taste.
Amantadine Hydrochloride is very soluble in formic acid, freely soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.

Identification (1) To 0.1 g of Amantadine Hydrochloride, add 1 mL of pyridine and 0.1 mL of acetic anhydride, dissolve by boiling for 1 minute, add 10 mL of dilute hydrochloric acid and cool in ice-water. Filter and collect the crystals separated, wash with water and dry at 105 ºC for 1 hour: the residue melts between 147 ºC and 151 ºC.

(2) Determine the infrared spectra of Amantadine Hydrochloride and amantadine hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Amantadine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Amantadine Hydrochloride in 5 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Amantadine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Amantadine Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Amantadine Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.50 g of Amantadine Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS and 10 mL of chloroform and shake. Filter the chloroform layer through absorbent cotton with 3 g of anhydrous sodium sulfate on a funnel and use the filtrate as the test solution. Pipet 1 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of each peak other than that of major peak from the test solution is not larger than 1/3 of the area of major peak from the standard solution and the total area of all peaks is not larger than the area of major peak from the standard solution.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 3 mm in internal diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with a mixture of branched hydrocarbon of petroleum hexamethyltetracosane group for gas chromatography and potassium hydroxide at the ratios of 2 % and 1 %, respectively.

Column temperature: Inject at a constant temperature of about 125 ºC, maintain the temperature for 5 minutes, raise at the rate of 5 ºC per minute to 150 ºC and maintain at a constant temperature of about 150 ºC for 15 minutes.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of amantadine is about 11 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of amantadine obtained from 2 μL of the standard solution composes about 10 % of the full scale.

System performance: Dissolve 0.15 g of naphthalene in 5 mL of the test solution and add chloroform to make 100 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, naphthalene and amantadine are eluted in this order with the resolution between these peaks being not less than 2.5.

Time span of measurement: About twice as long as the retention time of amantadine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

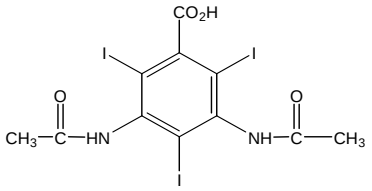
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.2 g of Amantadine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS and heat on a water-bath for 30 minutes. After cooling, add acetic acid (100) to make 70 mL and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.771 mg of $C_{10}H_{17}N \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Amidotrizoic Acid



$C_{11}H_9I_3N_2O_4$: 613.91

3,5-Diacetamido-2,4,6-triiodobenzoic acid [*117-96-4*]

Amidotrizoic Acid contains not less than 98.0 % and not more than 101.0 % of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), calculated on the dried basis.

Description Amidotrizoic Acid is a white, crystalline powder and is odorless.

Amidotrizoic Acid is slightly soluble in ethanol (95), very slightly soluble in water and practically insoluble in ether.

Amidotrizoic Acid dissolves in sodium hydroxide TS.

Identification (1) Heat 0.1 g of Amidotrizoic Acid over flame: a purple gas is evolved.

(2) Determine the infrared spectra of Amidotrizoic Acid and amidotrizoic acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Amidotrizoic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) ***Primary aromatic amines***—Dissolve 0.20 g of Amidotrizoic Acid in 5 mL of water and 1 mL of sodium hydroxides TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake and allow to stand for 2 minutes. Add 5

mL of ammonium sulfamate TS, shake well, allow to stand for 1 minute and add 0.4 mL of a solution of 1-naphthol in ethanol (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Determine the absorbance of this solution at 485 nm using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.15.

(3) ***Soluble halides***—Dissolve 2.5 g of Amidotrizoic Acid in 20 mL of water and 2.5 mL of ammonia TS, add 20 mL of dilute nitric acid and water to make 100 mL, allow to stand for 15 minutes with occasional shaking and filter. Discard the first 10 mL of the filtrate, transfer the subsequent 25 mL of the filtrate to a Nessler tube and add ethanol (95) to make 50 mL. Proceed as directed under the Chloride Limit Test using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 25 mL, then ethanol (95) to make 50 mL.

(4) ***Iodine***—Dissolve 0.20 g of Amidotrizoic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, add 5 mL of chloroform, shake well and allow to stand: the solution is colorless in the chloroform layer.

(5) ***Heavy metals***—Proceed with 2.0 g of Amidotrizoic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Arsenic***—Prepare the test solution with 0.6 g of Amidotrizoic Acid according to Method 3 and perform the test (not more than 3.3 ppm).

Loss on Drying Not more than 7.0 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

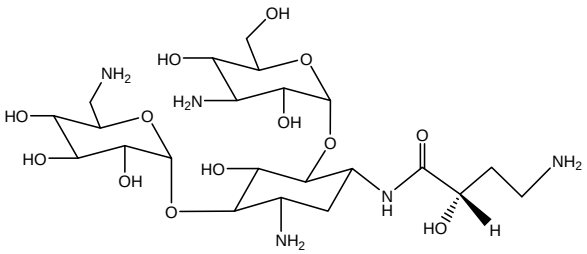
Assay Transfer about 0.5 g of Amidotrizoic Acid, accurately weighed, to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, connect to a reflux condenser, boil for 30 minutes, cool and filter. Wash the flask and the filter paper with 50 mL of water and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution and titrate with 0.1 mol/L silver nitrate VS until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS
= 20.464 mg of $C_{11}H_9I_3N_2O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Amikacin



$C_{22}H_{43}N_5O_{13}$: 585.60

(2*S*)-4-Amino-*N*-[(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-2-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4-{[(2*R*,3*R*,4*S*,5*S*,6*R*)-6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy}-3-hydroxycyclohexyl]-2-hydroxybutanamide [*37517-28-5*]

Amikacin contains not less than 900 μg (potency) of amikacin ($C_{22}H_{43}N_5O_{13}$) per mg, calculated on the anhydrous basis.

Description Amikacin is a white to pale grayish white wool-like powder.

Identification (1) Weigh about 60 mg each of Amikacin and Amikacin Sulfate RS, dissolve each in water to make the solutions so that each mL contains 6 mg, and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 3 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the developing solvent which is a mixture of methanol, ammonia solution (28), and chloroform (60:35:25) in a 230 x 230 x 90 mm developing chamber for 5 hours and 30 minutes, in which 200 x 30 mm Whatman filter paper or an identical filter paper strip is hung in the front of the chamber, the plate is slanted for the silica gel layer to face toward the front side of the chamber, and the cover with holes is placed on the top. The holes are sealed with scotch tape except the holes above the filter paper. Air-dry the plate, and spray evenly the mixture of 100 mL of 1 % ninhydrin·butanol solution and 1 mL of pyridine, and heat at 110 °C for 10 minutes: the spots obtained from the test solution and the standard solution is the same in the R_f value.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +97 ~ +105°(0.5 g calculated on the anhydrous basis, water, 25 mL, 100

mm).

pH The pH of a solution obtained by dissolving 0.1 g of Amikacin in 10 mL of water is between 9.5 and 11.5.

Water Not more than 8.5 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 1.0 % (1 g). Moisten the carbonized residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (1) ② under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633.

(3) Weigh accurately about 15 mg (potency) of Amikacin, and dissolve in sterile distilled water to make the solution so that each mL contains 400 μg (potency), and use the solution as the test stock solution. Take exactly a suitable amount of the test stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 15 mg (potency) of Amikacin Sulfate RS, dissolve in 0.05 mol/L phosphate buffer solution, pH 6.0 to make the solution so that each mL contains 400 μg (potency), and use the solution as the standard stock solution. Keep the standard stock solution between 5 °C and 15 °C, and use within 30 days. Take exactly a suitable amount of the standard stock solution in use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Amikacin Injection

Amikacin Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.61).

Method of Preparation Prepare into a liquid injection as directed under Injections, with Amikacin and with the aid of sulfuric acid.

Description Amikacin Injection appears as a clear and colorless to pale yellow liquid.

Identification Proceed as directed in the Identification under Amikacin.

pH 3.5 ~ 5.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.33 EU/mg (potency) of amikacin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

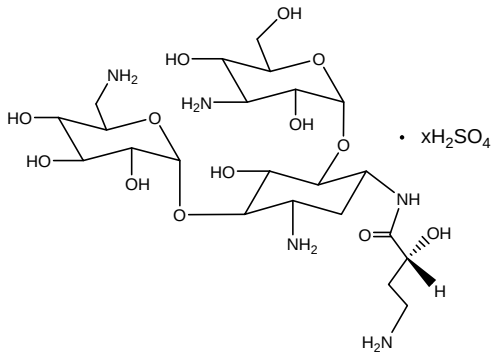
Assay ***The Turbidimetric method*** (1) Liquid medium for suspending the test organism- Use the culture medium in III 2 under Microbial Assay for Antibiotics.

(2) Test organism and test organism suspension- *Staphylococcus aureus* ATCC 6538P. Add 0.1 mL of the adjusted suspension to 100 m of the liquid medium for suspending the test organism, and use as the test organism suspension.

(3) Weigh accurately an amount of Amikacin Injection, add sterile purified water to make a solution of suitable concentration, pipet a suitable amount of this solution, dilute with sterile purified water so that each mL contains 10.0 μg (potency), and use this solution as the test solution. Separately, weigh accurately a suitable amount of Amikacin Sulfate RS, and dissolve in a sufficient amount of sterile purified water to make a standard stock solution so that each mL contains 1 mg (potency). Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 14 days. Pipet a suitable amount of the standard stock solution, dilute with sterile purified water so that each mL contains 8.0, 8.9, 10.0, 11.2, and 12.5 μg (potency), and use these solutions as the standard solutions. Perform the test with these solutions as directed in III 6 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Hermetic containers.

Amikacin Sulfate



$C_{22}H_{43}N_5O_{13}\cdot xH_2SO_4$

4-Amino-*N*-[5-amino-2-[4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-[6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy-3-hydroxycyclohexyl]-2-hydroxybutanamide; sulfuric acid [*39831-55-5*]

Amikacin Sulfate is the sulfate of a derivative of kanamycin.
Amikacin Sulfate contains not less than 691 μg (potency) and not more than 791 μg (potency) of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60) per mg, calculated on the dried basis.

Description Amikacin Sulfate appears as white to yellowish white powder.
Amikacin Sulfate is very soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the infrared spectra of Amikacin Sulfate and Amikacin Sulfate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometery: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g each of Amikacin Sulfate and Amikacin Sulfate RS in 4 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia solution (28), methanol, and tetrahydrofuran (1 : 1 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100 °C for 10 minutes: The spots obtained from the test solution and standard solution are red-purple in color and have the same R_f value.

(3) A solution of Amikacin Sulfate (1 in 100) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +76 ~ +84° (1 g, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Amikacin Sulfate in 10 mL of water is between 6.0 and 7.5.

Purity (1) ***Heavy metals***— Proceed with 1.0 g of Amikacin Sulfate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.1 g of Amikacin Sulfate in 4 mL of water, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia solution (28), methanol, and tetrahydrofuran (1 : 1 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100 °C for 10 minutes: the spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Sterility Test It meets the requirement, when Amikacin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg of amikacin, when Amikacin Sulfate is used in a sterile preparation.

Loss on Drying Not more than 4.0 % (0.1 g, in vacuum, 60 ºC, 3 hours).

Assay Weigh accurately an amount each of Amikacin Sulfate and Amikacin Sulfate RS, equivalent to about 50 mg (potency), and dissolve each in water to make exactly 50 mL. Pipet 200 μL each of these solutions into glass-stoppered test tubes, add exactly 3 mL of pyridine and exactly 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid dihdyrate (1 in 100), insert the stoppers, and warm in a water bath at 70 °C for 30 minutes. After cooling, add exactly 2 mL each of acetic acid (100), and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of amikacin derivative in each solution.

$$\text{Amount [μg (potency)] of amikacin } (C_{22}H_{43}N_5O_{13}) = \text{Amount [μg (potency)] of Amikacin Sulfate RS} \times \frac{H_T}{H_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 340 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 6.5 with a solution of potassium hydroxide (1 in 40), and add water to make 1000 mL. To 280 mL of this solution add 720 mL of methanol and mix.

Flow rate: Adjust the flow rate so that the flow rate of the amikacin derivative is about 9 minutes.

System suitability

System performance: Dissolve about 5 mg (potency) each of Amikacin Sulfate and kanamycin sulfate in 5 mL of water. Transfer 200 μL of this solution to a glass-stoppered test tube, add 3 mL of pyridine and 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid dihydrate (1 in 100), insert the stopper, and warm in a water bath at 70 °C for 30 minutes. After cooling, add 2 mL of acetic acid (100). When the procedure is run with 20 μL of this solution under the above operating conditions, amikacin derivative and kanamycin derivative are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of the amikacin derivative is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Amikacin Sulfate Injection

Amikacin Sulfate Injection is an aqueous injection.
Amikacin Sulfate Injection contains not less than 90.0 % and not more than 115.0 % of the labeled amount of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60).

Method of Preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate Injection appears as a clear and colorless to pale yellow liquid.

Identification To a volume of Amikacin Sulfate Injection, equivalent to 0.1 g (potency) of amikacin sulfate according to the labeled amount, add water to make 4 mL and use this solution as the test solution. Separately, dissolve an amount of Amikacin Sulfate RS, equivalent to 25 mg (potency), in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Amikacin Sulfate.

pH 6.0 ~ 7.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of amikacin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Amikacin Sulfate Injection, equivalent to about 0.1 g (potency) of amikacin sulfate according to the labeled amount, and add water to make exactly 100 mL. Separately, weigh accurately about 50 mg (potency) of Amikacin Sulfate RS, and add water to make exactly 50 mL. Pipet 200 μL each of these solutiosn into stoppered test tubes, add exactly 3 mL of pyridine and exactly 2 mL of a solution of 2,4,6-trinitribenzenesulfonic acid dihydrate (1 in 100), insert the stoppers, and warm in a water bath at 70 °C for 3 minutes. After cooling, add 2 mL of acetic acid (100), and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of the test solution and standard solution.

$$\text{Amount [mg (potency)] of amikacin } (C_{22}H_{43}N_5O_{13}) = \text{Amount [mg (potency)] of Amikacin Sulfate RS} \times \frac{H_T}{H_S} \times 2$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 340 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 6.5 with a solution of potassium hydroxide (1 in 40), and add water to make 1000 mL. To 280 mL of this solution add 720 mL of methanol and mix.

Flow rate: Adjust the flow rate so that the flow rate of the amikacin derivative is about 9 minutes.

System suitability

System performance: Dissolve about 5 mg (potency) of the test solution and about 5 mg of kanamycin sulfate in 5 mL of water. Transfer 200 μL of this

solution to a stoppered test tube, add exactly 3 mL of pyridine and 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid dihydrate (1 in 100), insert the stopper, and warm in a water bath at 70 °C for 30 minutes. After cooling, add 2 mL of acetic acid (100). When the procedure is run with 20 µL of this solution under the above operating conditions, amikacin derivative and kanamycin derivative are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of the amikacin derivative is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Aminocaproic Acid

$H_2NCH_2CH_2CH_2CH_2CH_2CO_2H$

$C_6H_{13}NO_2$: 131.17

6-Aminohexanoic acid [*60-32-2*]

Aminocaproic Acid contains not less than 98.5 % and not more than 101.5 % of aminocaproic acid ($C_6H_{13}NO_2$), calculated on the anhydrous basis.

Description Aminocaproic Acid is a fine white crystalline powder and is odorless.
Aminocaproic Acid is freely soluble in water, in acid, or in alkali, slightly soluble in methanol or in ethanol (95), and practically insoluble in chloroform or in ether.
A solution of Aminocaproic Acid is neutral.

Melting point—About 205 ºC.

Identification Determine the infrared spectra of Aminocaproic Acid and Aminocaproic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Heavy metals***—Proceed with 1.0 g of Aminocaproic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.12 g of Aminocaproic Acid and add water to make exactly 10 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution and dilute with water to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.12 g of Aminocaproic Acid RS, previously dried 105 ºC for 30 minutes, dissolve in water to make exactly 10 mL, then pipet 5 mL of this solution, add exactly 2 mL of internal standard solution and dilute with water to make 100 mL and use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Aminocaproic Acid to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of Aminocaproic Acid } (C_6H_{13}NO_2) = \text{Amount (mg) of Aminocaproic Acid RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 25 mg of methionine in 20 mL of water.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm to 10 µm in particle diameter).

Column temperature: A constant temperature of about 30 ºC.

Mobile phase: Dissolve 0.55 g of sodium 1-heptanesulphonate to make 1000 mL and use this solution as solution A. Dissolve 10 g of monobasic potassium phosphate in 300 mL of solution A, add 250 mL of methanol, then add 300 mL of solution A and mix. Adjust pH to 2.2 with phosphoric acid, and add solution A to make 1000 mL.

Flow rate: 0.7 mL/minute.

System suitability

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, Aminocaproic acid and methionine are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Aminocaproic Acid Tablets

Aminocaproic Acid Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of

aminocaproic acid ($C_6H_{13}NO_2$: 131.17).

Method of Preparation Prepare as directed under Tablets, with Aminocaproic Acid.

Identification Powder two Aminocaproic Acid Tablets, add 10 mL of water, shake well, with 100 mL of acetone and filter. Mix the filtrates with shaking and allow to stand for 15 minutes to be crystallized. Filter the crystals through a glass filter (G4) and wash with 25 mL of acetone and dry at 105 ºC for 30 minutes and cool. With the residue, proceed as directed in the Identification under Aminocaproic Acid.

Dissolution Test Perform the test with 1 tablet of Aminocaproic Acid Tablets at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of water as dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and use this solution as the test solution. Separately, weigh accurately sufficient quantity of Aminocaproic Acid RS, previously dried at 105 ºC for 30 minutes, dissolve in water, so that each mL contains about 500 μg of aminocaproic acid and use this solution as the standard solution. To 1 mL each of the test solution and the standard solution add 20.0 mL of borate buffer solution, pH 9.5, and 3.0 mL of sodium *β*-naphthoquinone-4-sulfonate TS, prepared before using, (1 in 500), shake, and allow to stand in a water-bath at 65 ± 5 ºC for 45 minutes. After cooling, add water to make exactly 50 mL and mix. Determine the absorbances of these solutions at 460 nm as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank. The dissolution rate of Aminocaproic Acid Tablets in 45 minutes is not less than 75 %.

Borate buffer solution, pH 9.5—Dissolve 6.185 g of boric acid and 7.930 g of potassium chloride in water, add 60 mL of 0.1 mol/L of sodium hydroxide VS and add water to make 2000 mL. Add 0.1 mol/L Sodium hydroxide, if necessary, to adjust to a pH of 9.5 ± 0.1.

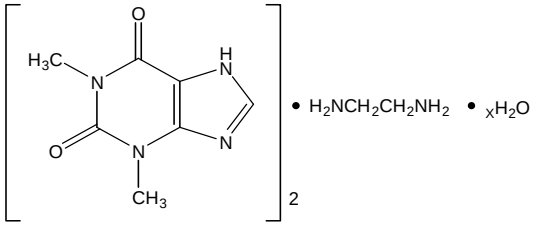
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Aminocaproic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of aminocaproic acid ($C_6H_{13}NO_2$), add 100 mL of acetic acid (100), dissolve by heating, cool and titrate with 0.1 mol/L perchloric acid in 1,4-dioxane VS until a blue color develops [indicator: 10 drops of chlorobenzene TS (1 in 500) of methylrozaniline chloride]. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L
perchloric acid in 1,4-dioxane VS
= 13.12 mg of $C_6H_{13}NO_2$

Containers and Storage ***Containers***—Tight containers.

Aminophylline Hydrate



$C_{14}H_{16}N_8O_4 \cdot C_2H_8N_2 \cdot xH_2O$

Bis(1,3-Dimethyl-2,3,6,7-tetrahydro-1*H*-purine-2,6-dione) ethane-1,2-diamine hydrate [*76970-41-7*]

Aminophylline Hydrate contains not less than 84.0 % and not more than 86.0 % of theophylline ($C_7H_8N_4O_2$: 180.16) and not less than 14.0 % and not more than 15.0 % of ethylenediamine ($C_2H_8N_2$: 60.10), calculated on the anhydrous basis.

Description Aminophylline Hydrate is a white to pale yellow granule or powder, is odorless or has slightly ammonia-like odor and has a bitter taste.
Aminophylline Hydrate is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) or in ether.
To 1 g of Amimophylline Hydrate, add 5 mL of water and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes and these crystals dissolve on the addition of a small amount of ethylenediamine
Aminophylline Hydrate is gradually colored by light and gradually loses ethylenediamine in air.

Identification (1) Dissolve 0.75 g of Aminophylline Hydrate in 30 mL of water and use this solution as the test solution. When 1 mL of dilute hydrochloric acid is added to 20 mL of the test solution, a precipitate is gradually formed. Filter and collect the precipitate, recrystallize from water and dry at 105 ºC for 1 hour: the crystals so obtained melt between 271 ºC and 275 ºC.

(2) Dissolve 0.1 g of the crystals obtained in (1) in 50 mL of water and to 2 mL of this solution, add tannic acid TS drop-wise: a white precipitate is produced and this precipitate dissolves upon drop-wise addition of tannic acid TS.

(3) To 10 mg of the crystals obtained in (1), add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid and evaporate on a water-bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a vessel containing 2 to 3

drops of ammonia TS: the color of the residue changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Dissolve 10 mg of the crystals obtained in (1) in 5 mL of water, add 3 mL of ammonia-ammonium chloride buffer solution, pH 8.0 and 1 mL of cupric sulfate-pyridine TS and mix. Add 5 mL of chloroform to the mixture and shake: the chloroform layer develops a green color.

(5) To 5 mL of the test solution obtained in (1), add 2 drops of copper (II) sulfate TS: a purple color develops. Add 1 mL of copper (II) sulfate TS: the color changes to blue and green precipitates are formed on staining.

pH Dissolve 1.0 g of Aminophylline Hydrate in 25 mL of water: the pH of this solution is between 8.0 and 9.5.

Purify (1) ***Clarity and color of solution***— Dissolve 1.0 g of Aminophylline Hydrate in 10 mL of hot water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Aminophylline Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 7.9 % (0.3 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay (1) ***Theophylline***—Weigh accurately about 0.25 g of Aminophylline Hydrate and dissolve in 50 mL of water and 8 mL of ammonia TS by gentle warming on a water-bath. Add exactly 20 mL of 0.1 mol/L silver nitrate VS, warm on a water-bath for 15 minutes, allow to stand between 5 ºC and 10 ºC for 20 minutes, collect the precipitate by suction and wash with three 10 mL portions of water. Combine the filtrate and washings and add dilute nitric acid to make neutral. Add 3 mL of dilute nitric acid and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 18.016 mg of $C_7H_8N_4O_2$

(2) ***Ethylenediamine***—Weigh accurately about 0.5 g of Aminophylline Hydrate, dissolve in 30 mL of water and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of bromphenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.0049 mg of $C_2H_8N_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Aminophylline Injection

Aminophylline Injection is an aqueous solution for injection. Aminophylline Injection contains not less than 75.0 % and not more than 86.0 % of theophylline ($C_7H_8N_4O_2$: 180.16) and not less than 13.0 % and not more than 20.0 % of ethylenediamine ($C_2H_8N_2$: 60.10) of the labeled amount of aminophylline. The concentration of Aminophylline Injection is expressed as the quantity of aminophylline hydrate ($C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$: 456.46).

Method of Preparation Prepare as directed under Injections, with Aminophylline hydrate. Aminophylline Injection may be prepared with Theophylline and its equivalent Ethylenediamine, instead of Aminophylline hydrate. Aminophylline Injection may contain not more than 60 mg of ethylenediamine as a stabilizer for each g of Aminophylline hydrate.

Description Aminophylline Injection is a clear and colorless liquid. Aminophylline Injection has a slightly bitter taste.
Aminophylline Injection gradually changes in color by light.

pH—8.0 ~ 10.0.

Identification To a volume of Aminophylline Injection, equivalent to 0.75 g of Aminophylline hydrate according to the labeled amount, add water to make 30 mL. Proceed with this solution as directed in the Identification under Aminophylline hydrate.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.6 EU/mg of aminophylline.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay (1) ***Theophylline***—Pipet an amount of Aminophylline Injection, equivalent to about 39.4 mg of theophylline ($C_7H_8N_4O_2$) (about 50 mg of aminophylline hydrate), add water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 40 mg of Theophylline RS, previously dried at 105 °C for 4 hours, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid

Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of theophylline in the test solution and the standard solution.

$$\text{Amount (mg) of theophylline } (C_7H_8N_4O_2) = \text{Amount (mg) of Theophylline RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4 : 1)

Flow rate: Adjust the flow rate so that the retention time of theophylline is about 5 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of theophylline are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of theophylline is not more than 1.0 %.

(2) ***Ethylenediamine***—To an accurately measured volume of Aminophylline Injection equivalent to about 30 mg of ethylenediamine ($C_2H_8N_2$)(about 0.2 g of Aminophylline hydrate), add water to make 30 mL and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 to 3 drops of bromphenol blue TS)

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.0049 mg of $C_2H_8N_2$

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Aminophylline Tablets

Aminophylline Tablets contain not less than 75.0 % and not more than 86.0 % of the labeled amount of theophylline ($C_7H_8N_4O_2$: 180.16) and not less than 12.0 % and not more than 14.0 % of the labeled amount of ethylenediamine ($C_2H_8N_2$: 60.10). The amount of Aminophylline Tablets is expressed as the quantity of aminophylline hydrate ($C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$: 456.46).

Method of Preparation Prepare as directed under Tablets, with Aminophylline Hydrate.

Identification (1) Weigh a portion of powdered Aminophylline Tablets, equivalent to 0.5 g of Aminophylline Hydrate according to labeled amount, add 25 mL of water, shake well, then filter. This filtrate changes moistened red litmus paper to blue. Mix the filtrate with 1 mL of 3 mol/L of hydrochloric acid, then obtain the crystals of Theophylline. Cool, if necessary, and precipitate. Filter and collect the precipitate (the filtrate is used for identification (2)), wash with small volume of cold water and dry at 105 ºC for 1 hour. Proceed with this crystal as directed in the Identification (3) under Aminophylline Hydrate. And recrystalize this crystal with water, dry at 105 ºC for 1 hour, and the melting point of this crystal is not less than 270 ºC and not more than 274 ºC.

(2) To the filtrate from (1), add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 mol/L sodium hydroxide TS to make alkaline, shake for 10 minutes, and then add 5 mL of 3 mol/L hydrochloric acid to make acidic, then obtain the crystals of ethylenediamine disulfonamide, wash, recrystalize, dry at for 105 ºC for 1 hour: the melting point of this crystals is not less than 164 ºC and not more than 171 ºC.

Dissolution Test This test applies to naked tablets. Perform the test with 1 tablet of Aminophylline Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water, as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test, filter, make any necessary dilution and use this solution as the test solution. Separately, weigh accurately about sufficient quantity of Aminophylline RS, previously dried at 105 ºC for 4 hours, make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Aminophylline Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay (1) ***Theophylline***—Weigh accurately and powder not less than 20 Aminophylline Tablets. Weigh accurately a portion of the powder, equivalent to about 2 g of aminophylline hydrate ($C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$), add 50 mL of water and 15 mL of ammonia TS, warm at 50 ºC and allow to stand for 30 minutes with occasional shaking. Cool to the room temperature and add water to make exactly 200 mL. Pipet 50 mL of this solution, centrifuge, pipet exact amount of the clear supernatant liquid, equivalent to 0.2 g of theophylline, into a conical flask and add water to make about 40 mL. Then add 8 mL of ammonia TS, add 20 mL of 0.1 mol/L silver nitrate VS, warm in water-bath for 15 minutes, then

cool at between 5 ºC and 10 ºC for 20 minutes, filter the precipitate through a glass filter (G4) and wash with three 10 mL volumes of water. Collect the filtrate and washings, add nitric acid to make acidic, then add 3 mL of nitric acid. After cooling, titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

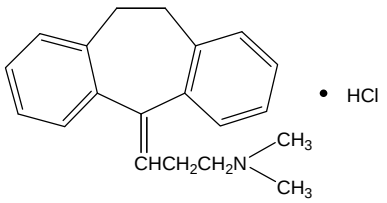
Each mL of 0.1 mol/L silver nitrate VS
= 18.016 mg of $C_7H_8N_4O_2$

(2) ***Ethylenediamine***—Weigh accurately and powder not less than 20 Aminophylline Tablets. Transfer a portion, weighed accurately, of the powder, equivalent to about 0.35 g of aminophylline hydrate ($C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$) into a conical flask, add 200 mL of water, digest at 50 ºC with frequent shaking for 30 minutes. After cooling, filter this solution to another conical flask, wash with water until the last washing is neutral to litmus and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 to 3 drops of methyl orange TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.0049 mg of $C_2H_8N_2$

Containers and Storage ***Containers***—Tight containers.

Amitriptyline Hydrochloride



$C_{20}H_{23}N \cdot HCl$: 313.86

N,*N*-Dimethyl-3-[tricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3(8),4,6,12,14-hexaen-2-ylidene]propan-amine hydrochloride [*549-18-8*]

Amitriptyline Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$).

Description Amitriptyline Hydrochloride appears as colorless crystals or a white to pale yellow crystalline powder. Amitriptyline Hydrochloride has a bitter taste and a numbing effect.
Amitriptyline Hydrochloride is freely soluble in water, in ethanol (95) or in acetic acid (100), soluble in acetic anhydride, and practically insoluble in ether.

pH—Dissolve 1.0 g of Amitriptyline Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 5.0.

Identification (1) Dissolve 5 mg of Amitriptyline Hydrochloride in 3 mL of sulfuric acid: a red color is observed. Add 5 drops of potassium bichromate TS to this solution: it turns dark brown.

(2) Acidify 1 mL of a solution of Amitriptyline Hydrochloride (1 in 500) with 0.5 mL of dilute nitric acid and add 1 drop of silver nitrate TS: a white precipitate is produced.

(3) Determine absorption spectra of solutions of Amitriptyline Hydrochloride and amitriptyline hydrochloride RS, in water (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 195 ~ 198 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Amitriptyline Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Amitriptyline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Weigh accurately a suitable amount of Amitriptyline Hydrochloride, dissolve in the mobile phase so that each mL contains 1 mg, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Dibenzosuberone RS, dissolve in methanol so that each mL contains 1 mg, and use this solution as standard stock solution (1). Separately, weigh accurately a suitable amount each of Amitriptyline Hydrochloride RS, Amitriptynol RS, Cyclobenzaprine Hydrochloride RS, and Nortriptyline Hydrochloride RS, dissolve in the mobile phase so that each mL contains 0.4 mg, 0.6 mg, 0.6 mg, and 0.6 mg, respectively, and use this solution as standard stock solution (2). Pipet suitable amounts of standard stock solutions (1) and (2), add the mobile phase to make a solution so that each mL contains 1 μg of amitriptyline hydrochloride, 0.5 μg of dibenzosuberone, 1.5 μg of amitriptynol, 1.5 μg of cyclobenzaprine hydrochloride, and 1.5 μg of nortriptyline hydrochloride, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the amount of each related substance by the following equation: dibenzosuberone is not more than 0.05 %, amitriptynol, nortriptyline, and cyclobenzaprine are not more than 0.15 %, respectively, each of the other related substances is not more than 0.10 %, and the total amount of related substances is not more than 1.0 %. Disregard any peaks with a relative retention time less than 0.22 with respect to amitriptyline.

Amount (%) of related substances

$$= 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

C_S: Concentration (mg/mL) of each specified related substance in the standard solution

C_T: Concentration (mg/mL) of amitriptyline hydrochloride in the test solution

A_T: Peak area of each specified related substance from the test solution

A_S: Peak area of each specified related substance from the standard solution

$$\text{Amount (\%) of each related substance} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration (mg/mL) of amitriptyline hydrochloride in the standard solution

C_T: Concentration (mg/mL) of amitriptyline hydrochloride in the test solution

A_i: Peak area of each related substance from the test solution

A_S: Peak area of amitriptyline hydrochloride from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column: A constant temperature of about 45 °C

Mobile phase: Dissolve 1.42 g of disodium hydrogen phosphate in 1000 mL of water, and adjust the pH to 7.7 with dilute phosphoric acid (1 in 10). To 300 mL of this solution add 700 mL of methanol.

Flow rate: About 1.5 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of amitriptynol and nortriptyline is not less than 1.5, and the relative retention times of dibenzosuberone, amitriptynol, nortriptyline hydrochloride, and cyclobenzaprine hydrochloride are about 0.35, about 0.52, about 0.60, and about 0.76, respectively, with respect to amitriptyline hydrochloride.

System repeatability: Weigh accurately a suitable amount of Amitriptyline Hydrochloride RS, and dissolve in the mobile phase to make a solution so that each mL contains 0.2 mg. When the test is repeated 6 times with this solution under the above operating conditions, the relative standard deviation of the peak areas of amitriptyline is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Amitriptyline Hydrochloride, previously dried and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.386 mg of $C_{20}H_{23}N{\cdot}HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Amitriptyline Hydrochloride Tablets

Amitriptyline Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N{\cdot}HCl$: 313.86).

Method of Preparation Prepare as directed under Tablets, with Amitriptyline Hydrochloride.

Identification (1) Weigh a portion of powdered Amitriptyline Hydrochloride Tablets, equivalent to 0.1 g of Amitriptyline Hydrochloride according to the labeled amount. Add 10 mL of chloroform, shake thoroughly and filter. Evaporate the filtrate on a water-bath to about 2 mL, add ether until turbidity is produced and allow to stand. Filter the crystals through a glass filter (G4) and proceed as directed under the Identification (1) and (2) under Amitriptyline Hydrochloride.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 238 nm and 240 nm and a minimum between 228 nm and 230 nm.

Dissolution Test Perform the test with 1 tablet of Amitriptyline Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of diluted phosphate buffer solution, pH 6.8 (1 in 2) as a dissolution solution. Take 20 mL or more of the dissolved solution 60 minutes after starting the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add diluted phosphate buffer solution, pH 6.8 (1 in 2), to make exactly V' mL, so that each mL contains about 11 μg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 55 mg of Amitriptyline Hydrochloride RS, previously dried at 105 °C for 2 hours and dissolve in diluted phosphate buffer solution, pH 6.8 (1 in 2) to make exactly 250 mL. Pipet 5 mL of this solu-

tion, add diluted phosphate buffer solution, pH 6.8 (1 in 2) to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 239 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Amitriptyline Hydrochloride Tablets in 60 minutes is not less than 70 %.

Dissolution rate (%) with respect to the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N\cdot HCl$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

W_S: Amount (mg) of Amitriptyline Hydrochloride RS
C: Labeled amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N\cdot HCl$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Amitriptyline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of amitriptyline hydrochloride ($C_{20}H_{23}N\cdot HCl$) and add mobile phase to make exactly 250 and filter. Discard the first 20 mL volume of the filtrate, and use next filtrate as the test solution. Separately, weigh accurately about 50 mg of Amitriptyline Hydrochloride RS, previously dried at 60 ºC, 0.67 kPa until the constant mass is attained and dissolve in mobile phase to make exactly 250 mL and use this solution as the standard solution. Perform the test with 20 µL each of the test solution an the standard solution as diretected under Liquid Chromatography according to the following conditions and calculate peak areas of amitriptyline, A_T and A_S, from the test solution and the standard solution, respectively,.

Amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N\cdot HCl$)
= Amount (mg) of Amitriptyline Hydrochloride RS

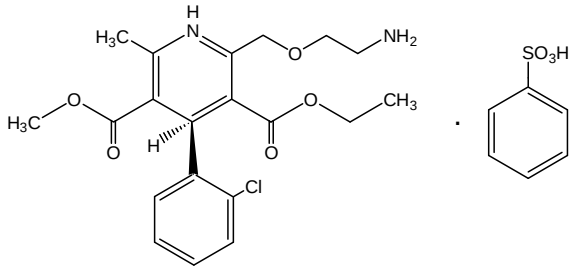
$$\times \frac{A_T}{A_S}$$

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (between 5 and 10 µm in particle diameter).
Mobile phase: A mixture of Liquid A and acetonitrile (58 : 42)
Liquid A: Dissolve 11.04 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 2.5 ± 0.5 with phosphoric acid, and add water to make 1000 mL.
Flow rate: 2 mL/minutes.
System suitability

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the symmetry factor of the peak of amitriptyline is not more than 2.0.
System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amitriptyline is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Amlodipine Besylate



and enantiomer

$C_{20}H_{25}N_2O_5Cl\cdot C_6H_6O_3S$: 567.05

3-Ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate [*111470-99-6*]

Amlodipine Besylate contains not less than 97.0 % and not more than 102.0 % of amlodipine besylate ($C_{20}H_{25}N_2O_5Cl\cdot C_6H_6O_3S$), calculated on the anhydrous basis.

Description Amlodipine Besylate is a white powder. Amlodipine Besylate is freely soluble in methanol, sparingly soluble in ethanol (95), and slightly soluble in water or 2-propanol.

Identification (1) To 30 mg of Amlodipine Besylate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, and filter if necessary. To the filtrate add barium chloride TS: a white precipitate is produced.
(2) Dissolve 5.0 mg each of Amlodipine Besylate and Amlodipine Besylate RS, respectively, in 1 vol % solution of 0.1 mol/L hydrochloric acid TS in methanol to make 100 mL. Determine the absorption spectra of the solutions respectively, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared spectra of Amlodipine Besylate and Amlodipine Besylate RS, previously

dried, as directed in the paste method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) The principal spot in the chromatogram obtained with the test solution (2) of i) *Related substances* is the same in color and R_f value as the principal spot with the standard solution (2).

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10º (0.25 g, methanol, 25 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Amlodipine Besylate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—i) Dissolve 0.140 g of the Amlodipine Besylate in methanol to make exactly 2 mL and use this solution as the test solution (1). To 1.0 mL of this solution add methanol to make exactly 10 mL and use this solution as the test solution (2). Separately, dissolve 70.0 mg of Amlodipine Besylate RS in 1.0 mL of methanol and use this solution as the standard solution (1). To 1.0 mL of the standard solution (1) add methanol to make 10 mL and use this solution as the standard solution (2). To 3.0 mL of the standard solution (2) add methanol to make 100 mL and use this solution as the standard solution (3). To 1.0 mL of the standard solution (2) add methanol to make 100 mL and use this solution as the standard solution (4). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solutions and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with upper layer of a mixture of 4-methyl-2-pentanone, water and acetic acid (100) (50 : 25 : 25) to a distance of about 15 cm and dry the plate at 80 °C for 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): any spot, other than the principal spot, in the chromatogram obtained with the test solution (1) is not more intense than that with the standard solution (3) (not more than 0.3 %) and more intense spot than the one in the chromatogram from the standard solution (4) is not more than 2 spots (not more than 0.1 %). This test is not valid unless the chromatogram from the standard solution (1) shows 2 clearly separated minor spots with R_f values of about 0.18 and 0.22.

ii) Dissolve 50.0 mg of Amlodipine Besylate in mobile phase to make exactly 50 mL and use this solution as the test solution (1). To 5.0 mL of the test solution (1) add mobile phase to make exactly 100 mL and use this solution as the test solution (2). Separately, dissolve 50.0 mg of Amlodipine Besylate RS in mobile phase to make exactly 50 mL, pipet 5.0 mL of this solution, add mobile phase to make exactly 100 mL and use this solution as the standard solution (1). To 3.0 mL of the test solution (1) add mobile phase to make exactly 100 mL, pipet 5.0 mL of this solution, add mobile phase to make exactly 50 mL and use this solution as the standard solution (2). Dissolve 5 mg of Amlodipine Besylate in 5 mL of strong hydrogen peroxide, heat at 70 °C for 45 minutes and use this solution as the standard solution (3). Perform the test with exactly 10 μL each of the test (1) and the standard solutions (2) and (3) as directed under Liquid Chromatography according to the following conditions: Amlodipine besylate related substance I {3-Ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl pyridine-3,5-dicarboxylate} obtained with the test solution is not more than the area of the principal peak in the chromatogram from the standard solution (2) (0.3 %). Total area of all the other related substances is not more than the area of the principal peak in the chromatogram from the standard solution (2) (0.3 %). Disregard any peak due to benzene sulfonate (relative retention : about 0.2) and 0.1 times the area of the principal peak with the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: Mix 15 volumes of acetonitrile, 35 volumes of methanol and 50 volumes of a solution prepared as follows: dissolve 7.0 mL of triethylamine in 1000 mL of water and adjust to pH 3.0 ± 0.1 with phosphoric acid.

Flow rate: 1.0 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (3) under the above operating conditions, minimum resolution is not less than 4.5 between the peaks corresponding to amlodipine and related substance I; relative retention time of related substance I with reference to amlodipine (retention time = about 7 minutes) is about 0.5. For the calculation of content, multiply the peak area of the related substance I by 2.

Time span of measurement: About 3 times as long as the retention time of amlodipine.

Water Not more than 0.5 % (3.0 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.2 % (1 g).

Assay Perform the test with the test solution (2) and the standard solution (1) of related substances II as directed above conditions in related substances II and calculate the percentage content of amlodipine besylate from the areas of the peaks, A_T and A_S, respectively.

$$\text{Amounts(mg) of amlodipine besylate } (C_{20}H_{25}N_2O_5Cl\cdot C_6H_6O_3S) = \text{Amounts (mg) of Amlodipine Besylate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Sorage—Light-resistant.

Ammonia Water

Azanium hydroxide

Ammonia Water contains not less than 9.5 w/v % and not more than 10.5 w/v % of ammonia (NH_3: 17.03)

Description Ammonia Water is a clear, colorless liquid and has a very pungent, characteristic odor.
Ammonia Water is alkaline.

Specific Gravity— d_{20}^{20} : 0.95 ~ 0.96.

Identification (1) Hold a glass rod moistened with hydrochloric acid near the surface of Ammonia Water: dense white fumes are produced.

(2) Hold moistened red litmus paper near the surface of Ammonia Water: it turns blue.

Purity (1) ***Residue on evaporation***—Evaporate 10.0 mL of Ammonia Water to dryness and dry the residue at 105 ºC for 1 hour: the weight of the residue is not more than 2.0 mg.

(2) ***Heavy metals***—Evaporate 5.0 mL of Ammonia Water to dryness on a water-bath, add 1 mL of dilute hydrochloric acid to the residue and evaporate to dryness. Dissolve the residue in 2 mL of dilute acetic acid, add water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of standard lead solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(3) ***Potassium permanganate-reducing substances***—To 10.0 mL of Ammonia Water, add 40 mL of dilute sulfuric acid while cooling and add 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color of the potassium permanganate does not disappear within 10 minutes.

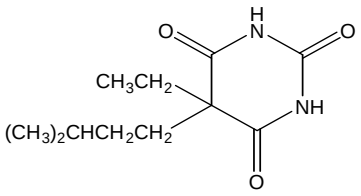
Assay Measure exactly 5 mL of Ammonia Water, add 25 mL of water and titrate with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 17.031 mg of NH_3

Containers and Storage ***Containers***—Tight containers.

Sorage—Not exceeding 30 ºC.

Amobarbital



$C_{11}H_{18}N_2O_3$: 226.27

5-Ethyl-5-(3-methylbutyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione [*57-43-2*]

Amobarbital, when dried, contains not less than 99.0 % and not more than 101.0 % of amobarbital ($C_{11}H_{18}N_2O_3$).

Description Amobarbital appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste.
Amobarbital is freely soluble in ethanol (95), in acetone or in ether, sparingly soluble in chloroform and practically insoluble in water.
Amobarbital dissolves in sodium hydroxide TS or sodium carbonate TS.

pH—The pH of a saturated solution of Amobarbital is between 5.0 and 5.6.

Identification (1) Boil 0.2 g of Amobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 50 mg of Amobarbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7 and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of cupric sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. Shake the mixture: a red-purple color is produced in the chloroform layer.

(3) To 0.4 g of Amobarbital, add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture in a water-bath for 30 minutes under a reflux condenser and allow to stand for 1 hour. Filter and collect the crystals produced, wash with 7 mL of sodium hydroxide TS and a small portion of water, recrystalize from ethanol (95) and dry at 105 ºC for 30 minutes: the crystals so obtained melt between 168 ºC and 173 ºC or between 150 ºC and 154 ºC.

Melting Point 157 ~ 160 ºC.

Purity (1) ***Clarity and color of solution***— Dissolve 0.5 g of Amobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.30 g of Amobarbital in 20 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this

solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid and add water to make 50 mL (not more than 0.035 %).

(3) ***Sulfate***—Dissolve 0.40 g of Amobarbital in 20 mL of acetone and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone and 1 mL of dilute hydrochloric acid and add water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Amobarbital according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Readily* carbonizable *substances***—Perform the test with 0.5 g of Amobarbital. The solution has no more color than Color Matching Fluid A.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 4 hours).

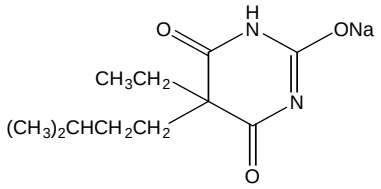
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Amobarbital, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through pale blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 22.627 mg of $C_{11}H_{18}N_2O_3$

Containers and Storage ***Containers***—Well-closed containers.

Amobarbital Sodium for Injection



$C_{11}H_{17}N_2NaO_3$: 248.25

Amobarbital Sodium for Injection is a preparation for injection which is dissolved before use. When dried, Amobarbital Sodium for Injection contains not less than 98.5 % and not more than 101.0 % of amobarbital sodium ($C_{11}H_{17}N_2NaO_3$) and not less than 92.5 % and not more than 107.5 % of the labeled amount of amobarbital sodium ($C_{11}H_{17}N_2NaO_3$).

Method of Preparation Prepare as directed under Injections with Amobarbital Sodium.

Description Amobarbital Sodium for Injection appears as white crystals or a crystalline powder, is odorless and has a bitter taste.
Amobarbital Sodium for Injection is freely soluble in water or in ethanol (95), and practically insoluble in ether or in chloroform.

pH— The pH of a solution of Amobarbital Sodium for Injection (1 in 10) is between 10.0 and 11.0.
Amobarbital Sodium for Injection is hygroscopic.

Identification (1) Dissolve 1.5 g of Amobarbital Sodium for Injection in 20 mL of water and add 10 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Collect the precipitate, wash with four 10mL volumes of water and dry at 105 ºC for 3 hours: it melts between 157 ºC and 160 ºC. With this precipitate, proceed as directed in the Identification under Amobarbital.

(2) Ignite 0.5 g of Amobarbital Sodium for Injection, cool and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests (1) for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Amobarbital Sodium for Injection in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 1.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake and filter. Discard the first 10 mL of the filtrate and to the subsequent 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.018 %).

(3) ***Sulfate***—Dissolve 2.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake and filter. Discard the first 10 mL of the filtrate and to the subsequent 25 mL of the filtrate, add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS, add 0.5 mL of acetic acid (100),1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019 %).

(4) ***Heavy metals***—Dissolve 2.0 g of Amobarbital Sodium for Injection in 45 mL of water, add 5 mL of dilute hydrochloric acid, shake vigorously and warm in a water-bath for 2 minutes with occasional shaking. Cool, add 30 mL of water, shake and filter. Discard the first 10 mL of the filtrate, add 1 drop of phenolphthalein TS to the subsequent 40 mL of the filtrate, add

ammonia TS until a slight red color develops and add 2.5 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.5 mL of dilute hydrochloric acid, add 1 drop of phenolphthalein, add ammonia TS until a pale red color develops and add 2.5 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 20 ppm).

(5) ***Neutral or basic substances***—Dissolve about 1 g of Amobarbital Sodium for Injection, accurately weighed, in 10 mL of water and 5 mL of sodium hydroxide TS, then add 40 mL of chloroform and shake well. Separate the chloroform layer, wash with two 5mL volumes of water and filter. Evaporate the filtrate on a water-bath to dryness and dry the residue at 105 ºC for 1 hour: the weight of the residue is not more than 0.30 %.

(6) ***Readily carbonizable substances***—Perform the test with 0.5 g of Amobarbital Sodium for Injection: the solution has no more color than Color Matching Fluid A.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.4 EU/mg of amobarbital sodium.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 10 samples of Amobarbital Sodium for Injection. Weigh accurately about 0.5 g of the contents, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid and extract with 50 mL of chloroform, then with three 25 mL volumes of chloroform. Combine the chloroform extracts, wash with two 5 mL volumes of water and extract the washings with two 10 mL volumes of chloroform. Filter the combined chloroform extracts into a conical flask and wash the filter paper with three 5 mL volumes of chloroform. Combine the filtrate and the washings and add 10 mL of ethanol (95). Titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through pale blue to purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination with a mixture of 160 mL of chloroform and 30 mL of ethanol (95) and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.825 mg of $C_{11}H_{17}N_2NaO_3$

Containers and Storage ***Containers***—Hermetic containers.

Amoxicillin Capsules

Amoxicillin Capsules contain not less than 92.0 % and not more than 105.0 % of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of Preparation Prepare as directed under Capsules, with Amoxicillin Hydrate.

Identification Take out the contents of Amoxicillin Capsules, weigh an amount of the contents, equivalent to 8 mg (potency) of amoxicillin hydrate according to the labeled amount, add 2 mL of 0.01 mol/L hydrochloric acid TS, shake for 30 minutes, filter, and use the filtrate as the test solution. Separately, dissolve an amount of Amoxicillin RS, equivalent to 8 mg (potency), in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, water, and formic acid (50 : 5 : 2) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 20) on the plate, and heat at 110 °C for 15 minutes: the principal spots obtained from the test solution and standard solution are red-purple in color and have the same R_f value.

Purity ***Related substances***—Take out the contents of Amoxicillin Capsules, weigh an amount of the contents, equivalent to 0.1 g (potency) of amoxicillin according to the labeled amount, add 30 mL of a solution of boric acid (1 in 200), shake for 15 minutes, and add a solution of boric acid (1 in 200) to make 50 mL. Centrifuge this solution, and use the clear supernatant liquid as the test solution. Pipet 1 mL of the test solution, add a solution of boric acid (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak other than amoxicillin obtained from the test solution is not larger than the peak area of amoxicillin from the standard solution.

Operating conditions

Proceed as directed in the operating conditions in the Purity (3) under Amoxicillin Hdyrate.

System suitability

Test for required detectability and system repeatability: Proceed as directed in the system suitability in the Purity (3) under Amoxicillin Hydrate.

System performance: When the procedure is run with 10 μL of the standard solution under the above

operating conditions, the number of theoretical plates and symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

Water Not more than 15.0 % (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Amoxicillin Capsules at 100 revolutions per minute according to Method 2 under Dissolution Test using the sinker, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 60 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg (potency) of amoxicillin ($C_{16}H_{19}N_3O_5S$), and use this solution as the test solution. Separately, weigh accurately an amount of Amoxicillin RS, equivalent to about 28 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin. The dissolution rate of Amoxicillin Capsules in 60 minutes is not less than 75 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of amoxicillin } (C_{16}H_{19}N_3O_5S) = \text{Amount [mg (potency)] of Amoxicillin RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

C: Labeled amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$) in 1 capsule.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

Column temperature: A constant temperature of about 25 °C.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of amoxicillin is not more than 1.5 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 20 Amoxicillin Capsules. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of amoxicillin hydrate according to the labeled potency, add 70 mL of water, shake for 15 minutes, add water to make exactly 100 mL, centrifuge, and use the clear supernatant liquid as the test solution. Separately, dissolve an amount of Amoxicillin RS, equivalent to about 20 mg (potency), in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in the test solution and standard solution.

$$\text{Amount [μg (potency)] of amoxicillin } (C_{16}H_{19}N_3O_5S) = \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of amoxicillin is not less than 2500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of amoxicillin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Amoxicillin for Syrup

Amoxicillin for Syrup is a preparation for syrup, which is dissolved before use.

Amoxicillin for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of Preparation Prepare as directed under Syrups, with Amoxicillin Hydrate.

Identification (1) Dissolve an amount of Amoxicillin for Syrup, equivalent to about 20 mg of amoxicillin, in 10 mL of water, and add 1 mL of Fehling's TS: a red-purple color develops immediately.

(2) To 2 mL of a solution of Amoxicillin for Syrup (1 in 5000) add 1 mL of a solution of mercury (II) sulfate in 2.5 mol/L sulfuric acid solution (3 in 20), heat on a water bath for 2 minutes, add 1 mL of a solution of sodium nitrite (1 in 1000), and heat for 2 minutes: a red-brown color develops.

(3) Weigh an amount of Amoxicillin for Syrup, equivalent to about 0.2 g of amoxicillin, and about 0.2 g of Amoxicillin RS, dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, water, and pyridine (9 : 8 : 3 : 1), and air-dry the plate. Spray evenly a solution, obtained by dissolving 0.3 g of ninhydrin in 100 mL of methanol, on the plate, and heat at 110 °C for 15 minutes: the spots obtained form the test solution and standard solution show the same R_f value.

pH The pH of a solution obtained by dissolving Amoxicillin for Syrup according to the label is between 5.0 and 7.5.

Water Not more than 3.0 % (0.2 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately an amount of Amoxicillin for Syrup, equivalent to about 0.1 g (potency) according to the labeled potency, dissolve in water, add 15.0 mL of the internal standard solution and water to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g (potency) of Amoxicillin RS, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquic Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amoxicillin to that of the internal standard in the test solution and standard solution.

Amount [μg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$)

$$= \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh accurately 0.7 g of sodium benzoate, and dissolve in water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1.5 minutes.

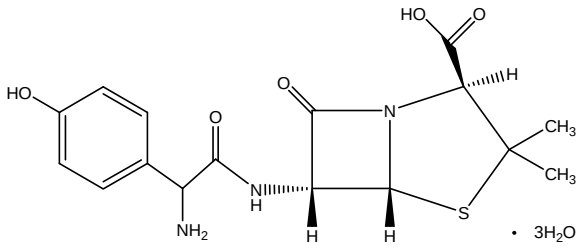
Mobile phase: Dissolve 6.3 g of ammonium formate in 750 mL of water, adjust the pH to 6.0 with formic acid or ammonia TS, add 30 mL of methanol, and add water to make exactly 1000 mL.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and amoxicillin are eluted in this order with the resolution between these peaks being not less than 2.0.

Containers and Storage ***Containers***—Tight containers.

Amoxicillin Hydrate



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$: 419.45

(3*S*)-6b-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2,2-dimethylpenam-3-carboxylic acid trihydrate [*61336-70-7*]

Amoxicillin Hydrate contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40), calculated on the anhydrous basis.

Description Amoxicillin Hydrate appears as white to pale yellowish white crystals or crystalline powder.
Amoxicillin Hydrate is slightly soluble in water or methanol, and very slightly soluble in ethanol (95).

Identification Determine the infrared spectra of Amoxicillin Hydrate and Amoxicillin RS as directed in

the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +290 ~ +315° (0.1 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 20 mg of Amoxicillin Hydrate in 10 mL of water is between 3.5 and 6.0.

Purity (1) ***Heavy metals***—To 1.0 g of Amoxicillin Hydrate add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), and heat on a water bath to dryness. Carbonize the residue by gently heating. After cooling, add 1 mL of sulfuric acid, heat carefully, then heat at 500 to 600 °C to incinerate. After cooling, add 1 mL of hydrochloric acid to the residue, and warm on a water bath to dryness. To the residue add 10 mL of water, and warm on a water bath to dissolve. After cooling, adjust the pH to 3 to 4 with ammonia TS, and add 2 mL of dilute acetic acid. If necessary, filter, wash with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), and proceed in the same manner as for preparation of the test solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Amoxicillin Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of sodium tetraborate decahydrate (1 in 200), and use this solution as the test solution. Pipet 1 mL of the test solution, add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than amoxicillin obtained from the test solution is not larger than the peak area of amoxicillin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in 750 mL of ater, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of amoxicillin is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of amoxicillin

(4) ***Dimethylaniline***—Weigh accurately about 1.0 g of Amoxicillin Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Amoxicillin Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for

gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C
Injection port and detector temperature: 150 °C
Carrier gas: Nitrogen
Flow rate: 30 mL/minute

Water 11.5 ~ 15.0 % (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 30 mg (potency) each of Amoxicillin Hydrate and Amoxicillin RS, dissolve each in a solution of boric acid (1 in 200) to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin.

$$\text{Amount [μg (potency)] of amoxicillin } (C_{16}H_{19}N_3O_5S_2) = \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

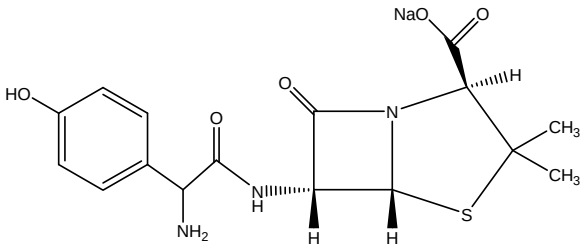
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of amoxicillin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Amoxicillin Sodium



$C_{16}H_{18}N_3NaO_5S$: 387.39

Sodium (3*S*)-6b-[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-2,2-dimethylpenam-3-carboxylate [*34642-77-8*]

Amoxicillin Sodium contains not less than 840 μg (potency) per mg of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40), calculated on the anhydrous basis.

Description Amoxicillin Sodium appears as white to milky white powder and is odorless or has a slight odor. Amoxicillin Sodium is very soluble in water or in methanol, soluble in ethanol (95), and practically insoluble in chloroform or in ether.

Identification (1) Determine the infrared spectra of Amoxicillin Sodium and Amoxicillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same numbers.

(2) Dissolve separately about 0.1 g (potency) each of Amoxicillin Sodium and Amoxicillin RS in 0.1 mol/L phosphate buffer solution (pH 7.0)[1)] to make 100 mL, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-methyl-1-propanol, methylene chloride, formic acid, and water (10 : 1 : 1 : 1). Warm the plate at 100 to 110 °C for 30 minutes, cool at room temperature, spray evenly a solution, obtained by dissolving 0.5 g of fast red GG in 100 mL of 0.1 mol/L sodium hydroxide TS, on the plate and develop the plate in a developing chamber saturated with 25 % ammonia water: an orange spot appears on a pale yellow background, and the spots obtained from the test solution and standard solution show the same R_f value.

(3) Amoxicillin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +255 ~ +285° (0.5 g calculated on the anhydrous basis, 0.02 mol/L potassium hydrogen phthalate solution, 50 mL)

pH The pH of a solution obtained by dissolving 0.3 g (potency) of Amoxicillin Sodium in 20 mL of water is between 8.5 and 9.5.

Purity (1) ***Clarity of solution***—Weigh accurately about 1 g (potency) of Amoxicillin Sodium, transfer to a 50 mL conical flask, add exactly 20 mL of purified water to dissolve, filter, and determine the absorbance at 425 nm of the filtrate after 5 minutes as directed under Ultraviolet-visible Spectrophotometry, using purified water as the blank (the absorbance is not more than 0.1).

(2) ***Heavy metals***—Proceed with 1.0 g of Amoxicillin Sodium according to Method 2 under Heavy Metals Limit Test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Iodine-consuming substances***—Weigh accurately about 0.1 g (potency) of Amoxicillin Sodium, transfer to a 50 mL glass-stoppered flask, add 5 mL of 0.05 mol/L potassium hydrogen phthalate TS and 20.0 mL of 0.005 mol/L iodine VS, and allow to stand in a dark place for 10 minutes. Titrate the excess iodine with 0.01 mol/L sodium thiosulfate VS (A mL) (indicator: 1 mL of starch TS). Separately, put 5 mL of 0.05 mol/L potassium hydrogen phthalate TS and 20.0 mL of 0.005 mol/L iodine VS into a 50 mL glass-stoppered flask, and, without allowing to stand, titrate the iodine with 0.01 mol/L sodium thiosulfate VS (B mL) (not more than 6.0 %).

$$\text{Content (\%) of iodine-consuming substances} = \frac{(B-A)\times 0.372}{\text{Amount (mg) of Amoxicillin Sodium taken}}\times 100$$

0.372: Amount (mg) of iodine-consuming substances corresponding to 1 ml of 0.01 mol/L sodium thiosulfate

(4) ***Chloride (as sodium chloride)***—Weigh accurately about 1 g of Amoxicillin Sodium, transfer to a 100 mL beaker, add 50 mL of distilled water and 5 mL of 4 mol/L nitric acid VS to dissolve, and titrate potentiometrically with silver nitrate TS (not more than 2.0 %).

$$\text{Content (\%) of chloride (as sodium chloride)} = \frac{A\times 5.84}{\text{Amount (mg) of Amoxicillin Sodium taken}}\times 100$$

A: Amount (mL) of silver nitrate TS consumed

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Amoxicillin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. PIpet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{\text{Amount (mg) of dimethylaniline taken}}{\text{Amount (mg) of Amoxicillin Sodium taken}}\times \text{Content (\%) of dimethylaniline}\times 4 = \frac{Q_T}{Q_S}\times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and 2 m in length, packed with silanized diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

(6) ***2-Ethylhexanoic acid***—Dissolve 0.3 g of Amoxicillin Sodium in 4.0 mL of 33 % hydrochloric acid solution, add 1.0 mL of the internal standard solution, shake vigorously for 1 minute, allow the layers to separate, and use the clear supernatant liquid as the test solution. Dissolve 75.0 g of 2-ethylhexanoic acid in the internal standard solution to make 50 mL, pipet 1.0 mL of this solution, add 4.0 mL of 33 % hydrochloric acid solution, shake vigorously for 1 minute, allow the layers to separate, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 2-ethylhexanoic acid to that of the internal standard: the amount of 2-ethylhexanoic acid is not more than 0.8 %.

$$\text{Amount (\%) of 2-ethylhexanoic acid} = \frac{Q_T}{Q_S}\times\frac{W_S}{W_T}\times 2$$

W_S: Amount (g) of Amoxicillin Sodium taken

W_T: Amount (g) of 2-ethylhexanoic acid in the standard solution

Internal standard solution—Weigh accurately 0.1 g of 3-cyclohexyl propinoic acid, and dissolve in cyclohexane to make 100 mL.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A glass column about 0.53 mm in internal diameter and about 10 m in length, the inside coated with polyethylene glycol 20000-2-nitroterephthalate for gas chromatography 1 μm in thickness.

Column temperature: Maintain at 40 °C for 2 minutes, raise the temperature to 200 °C at 7.3 minutes at the rate of 30 °C per minute, and maintain at 200 °C until 10.3 minutes.

Injection port temperature: 200 °C

Detector temperature: 300 °C

Carrier gas: helium

Flow rate: 10 mL/minute

System suitability

System performance: When the procedure is run with 1 μL each of the test solution and standard solution under the above operating conditions, 2-ethylhexanoic acid and the internal standard are eluted in this oder with the resolution between these peaks being not less than 2.0.

Water Not more than 3.5 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the reuiqrment, when Amoxicillin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.25 EU/mg (potency) of amoxicillin, when Amoxicillin Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.15 g (potency) of Amoxicillin Sodium, dissolve in 5 mL of 0.5 mol/L phosphate buffer solution (pH 7.0) and 5 mL of methanol, add water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 0.15 g (potency) of Amoxicillin RS, proceed in the same manner as for preparation of the test solution, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in the test solution and standard solution.

$$\text{Amount [μg (potency)] of amoxicillin } (C_{16}H_{19}N_3O_5S) = \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of 0.05 mol/L phosphate buffer solution (pH 4.5) and acetonitrile (85 : 15)

Containers and Storage ***Containers***—Tight containers.

Amoxicillin Sodium for Injection

Amoxicillin Sodium for Injection is a preparation for injection, which is dissolved before use.
Amoxicillin Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.41).

Method of Preparation Prepare as directed under Injections, with Amoxicillin Sodium.

Description Amoxicillin Sodium for Injection appears as white to milky white powder.

Identification Proceed with an amount of Amoxicillin Sodium for Injection, equivalent to 0.1 g (potency) of amoxicillin according to the labeled amount, and about 0.1 g (potency) of Amoxicillin RS as directed in the Identification (2) under Amoxicillin Sodium.

pH The pH of a solution obtained by dissolving an amount of Amoxicillin Sodium for Injection, equivalent to 1.5 g (potency) of amoxicillin, in 100 mL of water is between 8.5 and 9.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.25 EU/mg (potency) of amoxicillin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Water Not more than 3.5 % (0.2 g, volumetric titration, direct titration)

Assay Proceed as directed in the Assay under Amoxicillin Sodium. Weigh accurately an amount of Amoxicillin Sodium, equivalent to about 0.15 g (potency) according to the labeled potency, dissolve in 5 mL of 0.5 mol/L phosphate buffer solution (pH 7.0), add water to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Amoxicillin Sodium·Clavulanate Potassium for Injection

Amoxicillin Sodium·Clavulanate Potassium for Injection is a preparation for injection, which is dissolved before use.
Amoxicillin Sodium·Clavulanate Potassium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.41) and clavulanic acid ($C_8H_9NO_5$: 199.16).

Method of Preparation Prepare as directed under Injections, with Amoxicillin Sodium and Potassium Clavulanate.

Description Amoxicillin Sodium·Clavulanate Potassium for Injection appears as white to pale yellowish white powder.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving an amount of Amoxicillin Sodium·Clavulanate Potassium for Injection, equivalent to 1.0 g (potency) of amoxicillin, in 10 mL of water is between 8.0 and 10.0.

Water Not more than 4.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.25 EU/mg (potency) of amoxicillin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Amoxicillin Sodium·Clavulanate Potassium Tablets. Weigh accurately an amount of Amoxicillin Sodium·Clavulanate Potassium for Injection, equivalent to about 50 mg (potency) of amoxicillin according to the labeled potency, add about 70 mL of water, shake well for 60 minutes to dissolve, add water to make exactly 100 mL, filter if necessary, and use as the test solution. Separately, weigh accurately about 50 mg (potency) of Amoxicillin RS and about 10 mg (potency) of Clavulanic Acid RS, add about 70 mL of water, shake well for 60 minutes to dissolve, add water to make exactly 100 mL, and use this solution as the standard solution.

Containers and Storage ***Containers***—Hermetic containers.

Amoxicillin Tablets

Amoxicillin Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of Preparation Prepare as directed under Tablets, with Amoxicillin Hydrate.

Identification Powder Amoxicillin Tablets, and proceed as directed in the Identification under Amoxicillin for Syrup.

Water 50 mg (potency) tablets: not more than 9.0 % (0.1 g, volumetric titration, direct titration), 0.125 g (potency) tablets and 0.25 g (potency) tablets: not more than 13.0 % (0.1 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 tablet of Amoxicillin Tablets at 75 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make V' mL so that each mL contains about 45 μg (potency) of amoxicillin ($C_{16}H_{19}N_3O_5S$), and use this solution as the test solution. Separately, weigh accurately a suitable amount of Amoxicillin RS, dissolve in pH 5.0 buffer solution to make the same concentration as the test solution, and use this solution as the standard solution. Use the test solution and standard solution within 6 hours. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin. The dissolution rate of Amoxicillin Tablets in 30 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$) in 1 tablet]

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 300 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter). Use a stainless steel column 2 mm in diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography, as a guard column.

Mobile phase: Filter a mixture of pH 5.0 phosphate buffer solution and acetonitrile (3900 : 100) through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the mobile phase.

Flow rate: 0.7 mL/minute

Column temperature: A constant temperature of about 40 °C

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the capacity factor is 1.1 to 2.8, and the number of theoretical plates and symmetry factor are not less than 1700 and not more than 2.5, respectively.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of amoxicillin is not more than 1.5 %.

pH 5.0 Phosphate buffer solution—Dissolve 27.2 g of potassium dihydrogen phosphate in about 3000 mL of water. Adjust the pH to 5.0 ± 0.1 with 45 %(w/w) potassium hydroxide solution, and add water to make 4000 mL.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Amoxicillin Tablets. Weigh a portion of the powder, equivalent to about 0.1 g (potency) according to the labeled potency, add a solution of sodium borate (1 in 200), shake for about 10 minutes, add a solution of sodium borate (1 in 200) to make exactly 50 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 0.1 g (potency) of Amoxicillin RS, proceed in the same manner as for preparation of the test solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in the test solution and standard solution.

Amount [μg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$)

$$= \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 m)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (100), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of ethanol (95) and mix.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column such that the number of theoretical plates of the peak of amoxicillin is not less than 2500.

Containers and Storage ***Containers***—Tight containers.

Amoxicillin·Clavulanate Potassium for Syrup

Amoxicillin·Clavulanate Potassium for Syrup is a preparation for syrup, which is dissolved before use.

Amoxicillin·Clavulanate Potassium for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40) and clavulanic acid ($C_8H_9NO_5$: 199.16).

Method of Preparation Prepare as directed under Syrups, so that Amoxicillin·Clavulanate Potassium for Syrup contains Amoxicillin and Potassium Clavulanate in the ratio of 4 : 1 (potency), 7 : 1 (potency), or 14 : 1 (potency).

Identification The retention time of the principal peak obtained from the test solution corresponds to that of the principal peak from the standard solution, as obtained in the Assay.

pH The pH of a suspension obtained by dissolving Amoxicillin·Clavulanate Potassium for Syrup according to the label is between 4.0 and 6.0.

Water Not more than 8.0 % (if the ratio of amoxicillin to potassium clavulanate is 4 : 1 (potency)), not more than 9.0 % (if the ratio of amoxicillin to potassium clavulanate is 7 : 1 (potency)), not more than 11.0 % (if the ratio of amoxicillin to potassium clavulanate is 14 : 1 (potency)) (0.1 g, volumetric titration, direct

titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Amoxicillin·Clavulanate Potassium Tablets. Weigh accurately an amount of Amoxicillin·Clavulanate Potassium for Syrup, equivalent to about 50 mg (potency) of amoxicillin according to the labeled potency, add about 70 mL of water, shake well for 60 minutes to dissolve, add water to make exactly 100 mL, filter if necessary, and use as the test solution. Separately, weigh accurately about 50 mg (potency) of Amoxicillin RS and an amount of Clavulanic Acid RS [about 12.5 mg (potency) if the ratio is 4 : 1, about 7.1 mg (potency) if the ratio is 7 : 1, about 3.6 mg (potency) if the ratio is 14 : 1], add about 70 mL of water, shake well for 60 minutes to dissolve, add water to make exactly 100 mL, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Amoxicillin·Clavulanate Potassium Tablets

Amoxicillin·Clavulanate Potassium Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40) and clavulanic acid ($C_8H_9NO_5$: 199.16).

Method of Preparation Prepare as directed under Tablets, so that Amoxicillin·Clavulanate Potassium Tablets contain Amoxicillin and Potassium Clavulanate in the ratio of 2 : 1 (potency), 4 : 1 (potency), or 7 : 1 (potency).

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Water (1) Not more than 7.0 % if the ratio of amoxicillin to potassium clavulanate is 2 : 1 (potency),

(2) Not more than 9.0 % if the ratio of amoxicillin to potassium clavulanate is 4 : 1 (potency),

(3) Not more than 11.0 % if the ratio of amoxicillin to potassium clavulanate is 7 : 1 (potency) (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Amoxicillin·Clavulanate Potassium Tablets at 75 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately a suitable amount each of Amoxicillin RS and Clavulanic Acid RS, dissolve separately in water to make the same concentration as the test solution, and use these solutions as the standard solutions. Perform the test with 20 μL each of the test solution and standard solutions as directed in the Assay, and determine the peak areas, A_T and A_S, of amoxicillin and clavulanic acid. The dissolution rate of Amoxicillin·Clavulanate Potassium Tablets in 30 minutes is not less than 85 % (Q) for amoxicillin, and not less than 80 % (Q) for clavulanic acid.

Dissolution rate (%) with respect to the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) or clavulanic acid ($C_8H_9NO_5$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of amoxicillin or clavulanic acid in the standard solution

C: Labeled amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$) or clavulanic acid ($C_8H_9NO_5$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of not less than 10 Amoxicillin·Clavulanate Potassium Tablets, add a sufficient amount of water, shake, make a solution so that each mL contains 1.25 mg (potency) of amoxicillin according to the labeled potency, filter if necessary, and use as the test stock solution. Pipet 40 mL of the test stock solution, add water to make 100 mL, and use this solution as the test solution. Use the test solution within 1 hour. Separately, weigh accurately about 50 mg (potency) of Amoxicillin RS and an amount of Clavulanic Acid RS [about 25 mg (potency) if the ratio is 2 : 1, about 12.5 mg (potency) if the ratio is 4 : 1, about 7.1 mg (potency) if the ratio is 7 : 1], add about 70 mL of water, shake well for 60 minutes to dissolve, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_{T1}, A_{S1}, A_{T2}, and A_{S2}, of amoxicillin and clavulanic acid in the test solution and standard solution.

Amount [μg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$)

$$= \text{Amount [μg (potency)] of Amoxicillin RS} \times \frac{A_{T1}}{A_{S1}}$$

Amount [μg (potency)] of clavulanic acid ($C_8H_9NO_5$)

$$= \text{Amount [μg (potency)] of Clavulanic Acid RS} \times \frac{A_{T2}}{A_{S2}}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Filter a mixture of pH 4.4 phosphate buffer solution and methanol (95 : 5) through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as as the mobile phase.

Flow rate: 2 mL/minute

System suitability

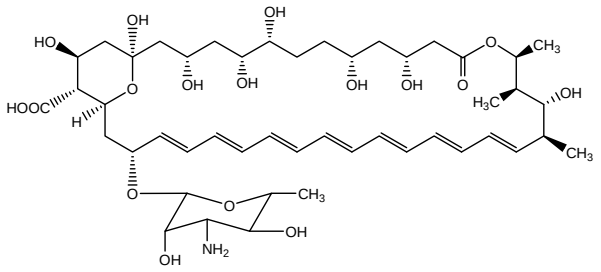
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of amoxicillin and clavulanic acid is not less than 3.5, and the number of theoretical plates and symmetry factor are not less than 550 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

pH 4.4 Phosphate buffer solution—Dissolve 7.8 g of sodium diihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 4.4 ± 0.1 with phosphoric acid and 10 mol/L sodium hydroxide solution, and add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Amphotericin B



$C_{47}H_{73}NO_{17}$: 924.08

(1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [*1397-89-3*]

Amphotericin B is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces nodosus*.

Amphotericin B contains not less than 840 μg (potency) of amphotericin B ($C_{47}H_{73}NO_{17}$) per mg, calculated on the dried basis.

Description Amphotericin B is a yellow to orange powder.

Amphotericin B is soluble in dimethylsulfoxide, and practically insoluble in water or in ethanol (95).

Identification (1) Dissolve 5 mg of Amphotericin B in 10 mL of dimethylsulfoxide. To 1 mL of this solution add 5 mL of phosphoric acid: a blue color develops between the two layers, and the solution becomes blue by shaking. After addition of 15 mL of water the solution becomes yellow to pale yellow-brown by shaking.

(2) Dissolve separately about 25 mg each of Amphotericin B and Amphotericin B RS in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL each of these solutions add methanol to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH The pH of a suspension obtained by suspending 0.3 g of Amphotericin B in 10 mL of water is between 3.5 and 6.0.

Purity ***Amphotericin A***—Weigh accurately about 50 mg each of Amphotericin B and Amphotericin B RS, dissolve each in exactly 10 mL of dimethylsulfoxide, and add methanol to make exactly 50 mL. Pipet 4 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the test solution and standard solution (1), respectively. Separately, weigh accurately about 20 mg of Nystatin RS, dissolve in 40 mL of dimethylsulfoxide, and add methanol to make exactly 200 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Determine the absorbances at 282 nm and 304 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution obtained in the same manner as the test solution without addition of Amphotericin B as the blank. Calculate the amount of amphotericin B by the following equation: not more than 5 % for amphotericin A used for injections, and not not more than 15 % for amphotericin A not used for injections.

$$\text{Amount (\%) of amphotericin A} = \frac{W_S \times \{(A_{Sa1} \times A_{T2}) - (A_{Sa2} \times A_{T1})\} \times 25}{W_T \times \{(A_{Sa1} \times A_{Sb2}) - (A_{Sa2} \times A_{Sb1})\}}$$

W_S: Amount (mg) of Nystatin RS

W_T: Amount (mg) of Amphotericin B (mg)

A_{Sa1}: Absorbance at 282 nm of the standard solution (1)

A_{Sb1}: Absorbance at 282 nm of the standard solution (2)

A_{Sa2}: Absorbance at 304 nm of the standard solution (1)
A_{Sb2}: Absorbance at 304 nm of the standard solution (2)
A_{T1}: Absorbance at 282 nm of the test solution
A_{T2}: Absorbance at 304 nm of the test solution

Loss on Drying Not more than 5.0 % (0.1 g, in vacuum, 60 °C, 3 hours)

Residue on Ignition Not more than 0.5 % (1 g)

Sterility Test It meets the requirement, when Amphotericin B is used in a sterile preparation.

Bacterial Endotoxins Less than 1.0 EU/mg (potency) of amphotericin B, when Amphotericin B is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) of Amphotericin B according to the labeled potency, and dissolve in water so that each mL contains 5 mg (potency). Pipet a suitable amount of this solution, add the mobile phase so that each mL contains 8 μg (potency), and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Amphotericin B RS, dissolve in methanol so that each mL contains 0.2 mg (potency), and use this solution as the standard stock solution. Pipet a suitable amount of the standard stock solution, add the mobile phase so that each mL contains 8 μg (potency), and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of amphotericin B in the test solution and standard solution.

Amount [μg (potency)] of amphotericin B ($C_{47}H_{73}NO_{17}$) = Amount [μg (potency)] of Amphotericin B RS $\times \frac{A_T}{A_S}$

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 405 nm)
Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Mobile phase: A mixture of 10 mol/L potassium phosphate solution and acetonitrile (66 : 34) (pH 4.8 ± 0.2)
Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.
Sorage—Light-resistant, and in a cold place.

Amphotericin B for Injection

Amphotericin B for Injection is a preparation for injection, which is dissolved before use.
Amphotericin B for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of amphotericin ($C_{47}H_{73}NO_{17}$: 924.08).

Method of Preparation Prepare as directed under Injections, with Amphotericin B.

Description Amphotericin B for Injection appears as yellow to orange powder or masses.

Identification Dissolve 25 mg (potency) of Amphotericin B for Injection in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 50 mL, and filter if necessary. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm, and between 403 nm and 407 nm.

pH The pH of a solution obtained by dissolving an amount of Amphotericin B for Injection, equivalent to 1 mg (potency) of amphotericin B, in 10 mL of water is between 7.2 and 8.0. For colloidal injections, the pH of a solution obtained by dissolving an amount, equivalent to 0.1 g (potency), in 20 mL of water is between 6.0 and 7.8.

Purity ***Clarity and color of solution***—Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of amphotericin B according to the labeled amount, in 10 mL of water: the solution is clear and yellow to orange.

Loss on Drying Not more than 8.0 % (0.3 g, in vacuum, 60 °C, 3 hours). For colloidal dispersion injections, not more than 2.5 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 3.0 EU/mg (potency) of amphotericin B.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Amphotericin B.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant, and in a cold place.

Ampicillin Capsules

Ampicillin Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of ampicililn ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Capsules, with Ampicillin.

Identification (1) Suspend an amount of the contents of Ampicillin Capsules, equivalent to about 10 mg of ampicillin, in 1 mL of water, and add 2 mL of a mixture of 2 mL of Fehling's TS and 6 mL of water: a red-purple color is produced immediately. For Ampicillin Capsules containing probenecid, wash a suitable amount of Ampicillin Capsules with chloroform, discard the chloroform layer, and perform the test with the residue.

(2) To 2 mL of a solution of Ampicillin Capsules, containing 1 mg of ampicillin per mL, add 0.5 mL of phenol and 5 mL of sodium hypochlorite TS: a persistent odor of benzaldehyde is perceptible, and an orange precipitate is produced within 3 to 5 minutes. For Ampicillin Capsules containing probenecid, wash a suitable amount of Ampicillin Capsules with chloroform, discard the chloroform layer, and perform the test with the residue.

Water Anhydride: Not more than 4.0 %, Hydrate: 10.0 ~ 15.0 % (0.1 g, volumetric titration, direct titration)

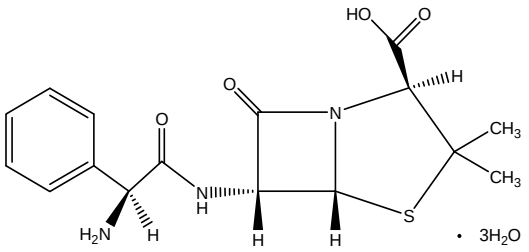
Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Ampicillin Hydrate. Weigh accurately the contents of not less than 20 Ampicillin Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Ampicillin Hydrate



$C_{16}H_{19}N_3O_4S \cdot 3H_2O$: 403.45

(3*S*,5*R*,6*R*)-6-[(2R)-2-amino-2-phenylacetamido]-2,2-dimethylpenam-3-carboxylic acid trihydrate [*7177-48-2*]

Ampicillin Hydrate contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41), calculated on the anhydrous basis.

Description Ampicillin. Hydrate appears as white to pale yellowish white crystals or crystalline powder.
Ampicillin Hydrate is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared spectra of Ampicillin Hydrate and Ampicillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +280 ~ +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Ampicillin Hydrate in 400 mL of water is between 3.5 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Ampicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Ampicillin Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50 mg of Ampicillin Hydrate in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions,

and determine the peak areas of each solution by the automatic integration method: the area of each peak other than ampicillin from the test solution is not larger than the peak area of ampicillin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the standard solution.

System performance: Weigh accurately 50 mg (potency) of Ampicillin RS, dissolve in 10 mL of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), add the mobile phase to make 50 mL, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicilin to that of guaifanesin is not more than 1.0 %.

Time span of measurement: About 10 times as long as the retention time of ampicillin

(4) ***Dimethylaniline***—Weigh accurately about 1.0 g of Ampicillin Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Ampicillin Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of ths solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water 12.0 ~ 15.0 % (0.1 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Ampicillin Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of ampicillin, when Ampicillin Hydrate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Ampicillin Hydrate and Ampicillin RS, dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = \text{Amount [μg (potency)] of Ampicillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

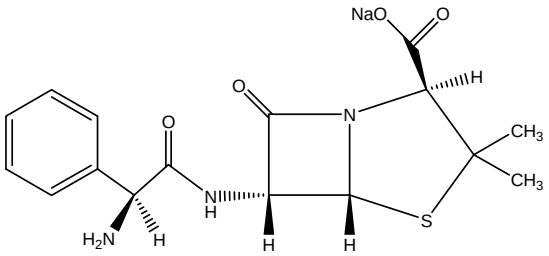
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicilin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Ampicillin Sodium



$C_{16}H_{18}N_3NaO_4S$: 371.39

Sodium (3*S*,5*R*,6*R*)-6-[(2R)-2-amino-2-phenylacetamido]-2,2-dimethylpenam-3-carboxylate [*69-52-3*]

Ampicillin Sodium contains not less than 850 μg (potency) and not more than 950 μg (potency) per mg of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41), calculated on the anhydrous basis.

Description Ampicillin Sodium appears as white to pale yellowish white crystals or crystalline powder.
Ampicillin Hydrate is very soluble in water, and sparingly soluble in ethanol (99.5).

Identification (1) Determine the infrared spectra of Ampicillin Sodium and Ampicillin Sodium RS, previously dried in a desiccator (reduced pressure not exceeding 0.67 kPa, 60 °C) for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Ampicillin Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement, except for freeze-dried ampicillin sodium.

Specific Optical Rotation $[\alpha]_D^{20}$: +246 ~ +272° (1.0 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Ampicillin Sodium in 10 mL of water is between 8.0 and 10.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ampicillin Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Ampicillin Sodium in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of each peak other than ampicillin from the test solution is not larger than the peak area of ampicilin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicil-

lin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the standard solution.

System performance: Weigh accurately 50 mg of Ampicillin RS, dissolve in 10 mL of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), add the mobile phase to make 50 mL, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin ot that of guaifenesin is not more than 1.0 %.

Time span of measurement: About 10 times as long as the retention time of ampicillin

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Ampicillin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Ampicillin Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of ths solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 2.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Ampicillin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of ampicillin, when Ampicillin Sodium is used in a sterile preparation.

Assay Perform the test according to Assay in Ampicillin Hydrate. Weigh accurately about 50 mg (potency) of Ampicillin Sodium, dissolve in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the test solution.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicililn and the internal standard are eluted in this order with the resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Ampicillin Sodium for Injection

Ampicillin Sodium for Injection is a preparation for injection, which is dissolved before use.
Ampicillin Sodium for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Injections, with Ampicillin Sodium.

Description Ampicillin Sodium for Injection appears as pale yellowish white crystals or crystalline powder.

Identification Proceed as directed in the Identification under Ampicillin Hydrate.

Osmotic Pressure Ratio It meets the requirement.

pH The pH of a solution obtained by dissolving an amount of Ampicillin Sodium for Inejction, equivalent to 1.0 g (potency) of ampicillin, in 10 mL of water is between 8.0 and 10.0.

Purity ***Clarity of solution***—Dissolve an amount of Ampicillin Sodium, equivalent to 0.25 g (potency) of ampicillin sodium, in 0.75 mL of water: the solution is

clear and its absorbance at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.40.

Water Not more than 3.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.075 EU/mg (potency) of ampicillin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ampicillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, add exactly 5 mL of the internal standard solution and the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Ampicillin Sodium RS, proceed in the same manner as the test solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = \text{Amount [μg (potency)] of Ampicillin Sodium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 5.9 g of ammonium monohydrogen phosphate add 850 mL of water and 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

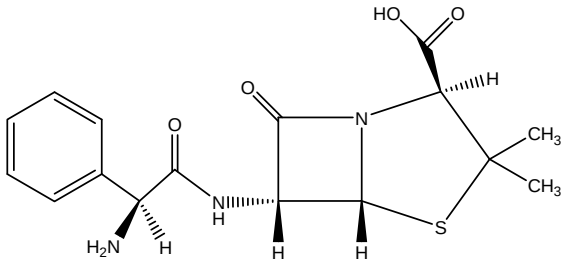
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 26.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Anhydrous Ampicillin



$C_{16}H_{19}N_3O_4S$: 349.40

(3*S*,5*R*,6*R*)-6-[(2R)-2-Amino-2-phenylacetamido]-2,2-dimethylpenam-3-carboxylic acid [*69-53-4*]

Anhydrous Ampicillin contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40), calculated on the anhydrous basis.

Description Anhydrous Ampicillin appears as white to pale yellowish white crystals or crystalline powder. Anhydrous Ampicillin is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared spectra of Anhydrous Ampicillin and Ampicillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +280 ~ +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Anhydrous Ampicillin in 100 mL of water is between 4.0 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.05 g of Anhydrous Ampicillin in the mobile phase to make 50 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than ampicillin from the test solution is not larger than the peak area of ampicillin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: Proceed as directed in the Assay.

System repeatability: Proceed as directed in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin

(4) ***Dimethylaniline***—Weigh accurately about 1.0 g of Anhydrous Ampicillin, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

$$\text{Amount (ppm) of dimethylaniline} = \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Purity (\%) of dimethylaniline}}{\text{Amount (mg) of Anhydrous Ampicillin taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of ths solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with silanized diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 2.0 % (2.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Anhydrous Ampicillin is used in a sterile preparation.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of ampicillin, when Anhydrous Ampicillin is used in a sterile preparation.

Assay Weigh accurately about 50 mg each of Anhydrous Ampicillin and Ampicillin RS, dissolve separately in exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = \text{Amount [μg (potency)] of Ampicillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

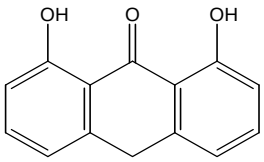
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicilin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Anthralin



Dithranol $C_{14}H_{10}O_3$: 226.23

1,8-Dihydroxy-10*H*-anthracen-9-one [*1143-38-0*]

Anthralin contains not less than 97.0 % and not more than 102.0 % of anthralin ($C_{14}H_{10}O_3$), calculated on the dried basis.

Description Anthralin is a yellow powder.
Anthralin is odorless and tasteless.
Anthralin is freely soluble in acetone, in chloroform, or in benzene, sparingly soluble in ethanol (95), in ether, or in acetic acid (100), and practically insoluble in water.
Anthralin is dissolves in sodium hydroxide TS

Identification (1) Determine the absorption spectra of solutions of Anthralin and Anthralin RS in chloroform (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Anthralin and Anthralin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the same intensities of absorption at the same wavenumbers.

Melting Point 178 ~ 181 ºC (Method 1).

Purity (1) ***Acid or base***—Suspend Anthralin in water and filter: the filtrate is neutral using Litmus paper.

(2) ***Chloride***—Suspend approximately 1.0 g of Anthralin in 15 mL of water and filter. Collect 5 mL of the filtrate, make acidic with nitric acid and add 2 to 3 drops of silver nitrate TS: the turbidity of this solution is not more than that of 5 mL of the filtrate.

(3) ***Sulfate***—Collect 5 mL of filtrate from (2), add 3 drops of 3 mol/L hydrochloric acid and 5 drops of barium chloride TS: the turbidity of this solution is not more than that of 5 mL of the filtrate.

Loss on Drying Not more than 0.5 % (1 g, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Anthralin, dissolve in dichloromethane to make exactly 100 mL, pipet 10 mL of this solution and add dichloromethane to make exactly 100 mL. Pipet 5 mL of this solution, add 5.0 mL of the internal standard solution and the mobile phase to make exactly 25 mL and use this solution as the test solution. Separately weigh accurately a portion of Anthralin RS, dissolve in dichloromethane to make 250 μg/mL. Pipet 5 mL of this solution, add 5.0 mL of the internal standard solution and the mobile phase to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Anthralin to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of Anthralin } (C_{14}H_{10}O_3) = C \times \frac{Q_T}{Q_S}$$

C: Concentration (μg/mL) of the standard solution.

Internal standard solution—Weigh a sufficient amount of *o*-nitroanilin, dissolve in a small volume of dichloromethane and add *n*-hexane to make 500 μg/mL.

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 354 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and 25 cm in length, packed with porous silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture of *n*-hexane, dichloromethane and acetic acid (100) (82 : 12 : 6).

Flow rate: 2 mL/minute.

System suitability

System performance: Dissolve 10 mg of Anthralin RS and 20 mg of danthrone in and dilute with dichloromethane to make 100 mL. To 5 mL of this solution add 5 mL of *n*-hexane and mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, anthralin and danthrone are eluted in this order with the

resolution between these peaks being not less than 1.3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area to that of the internal standard.is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Anthralin Ointment

Dithranol Ointment

Anthralin Ointment contains not less than 90.0 % and not more than 115.0 % of the labeled amount of anthralin ($C_{14}H_{10}O_3$: 226.23) when labeled amount of Anthralin in Anthralin Ointment is not less than 0.1 %. Anthralin Ointment contains not less than 90.0 % and not more than 130.0 % of the labeled amount of anthralin ($C_{14}H_{10}O_3$: 226.23) when the labeled amount of Anthralin in Anthralin Ointment is not more than 0.1 %.

Method of Preparation Prepare as directed under the Ointments with Anthralin.

Identification Retention time of major peak of the test solution from Assay is the same as that of the standard solution.

Assay Weigh accurately about 5 g of Anthralin Ointment in 100 mL of beaker and disperse with 20 mL of dichloromethane and 10 mL of acetic acid (100). Filter the content with dichloromethane using Whatman filter paper No. 4 to 100 mL volumetric flask. Wash the precipitate with dichloromethane to make 100 mL. Weigh accurately about 0.5 mg of anthralin ($C_{14}H_{10}O_3$), add 2.0 mL of the internal standard solution and the mobile phase to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately a portion of Anthralin RS and dissolve in dichloromethane to make 0.25 mg/mL. Pipet 2 mL of this solution, add 2.0 mL of the internal standard solution and the mobile phase to make exactly 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Assay of Anthralin.

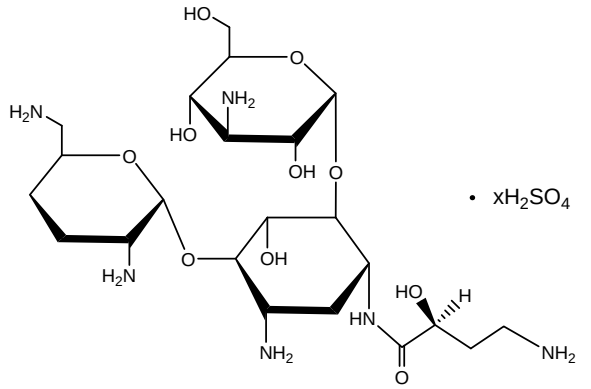
$$\text{Amount(mg) of Anthralin } (C_{14}H_{10}O_3) = 200 \times \frac{C}{V} \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of the standard solution.
V: Volume (mL) of the test solution collected.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Arbekacin Sulfate



$C_{22}H_{44}N_6O_{10}{\cdot}xH_2SO_4$ (x= 2 – 2½)

(2*S*)-4-Amino-*N*-[(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-4-[(2*R*,3*R*,6*S*)-3-amino-6-(aminomethyl)oxan-2-yl]oxy-2-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3-hydroxycyclohexyl]-2-hydroxybutanamide sulfate [*104931-87-5*]

Arbekacin Sulfate is the sulfate of a derivative of dibekacin.
Arbekacin Sulfate contains not less than 670 μg (potency) and not more than 750 μg (potency) per mg of arbekacin ($C_{22}H_{44}N_6O_{10}$: 552.62), calculated on the dried basis.

Description Arbekacin Sulfate appears as white powder.
Arbekacin Sulfate is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve separately 10 mg each Arbekacin Sulfate and Arbekacin Sulfate RS in 1 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for liquid chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform, and ethanol (95) (7 : 6 : 4 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate and heat for 10 minutes: the principal spot obtained from the test solution and the spot from the standard solution are purple-brown in color and have the same R_f value.

(2) A solution of Arbekacin Sulfate (1 in 50) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +69 ~ +79° (0.25 g after drying, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving about 0.75 g (potency) of Arbekacin Sulfate in 10 mL of water is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Arbekacin Sulfate in 5 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Arbekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Dibekacin***—Weigh accurately about 20 mg of Arbekacin Sulfate, dissolve in exactly 10 mL of the internal standard solution, add water to make 20 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Dibekacin Sulfate RS, equivalent to about 10 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dibekacin to that of the internal standard. Calculate the amount of dibekacin by the following equation: not more than 2.0 %.

$$\text{Amount (\%) of dibekacin} = \frac{\text{Amount [mg (potency)] of Dibekacin Sulfate RS}}{W_T} \times \frac{Q_T}{Q_S} \times \frac{1}{50} \times 100$$

W_T: Amount (mg) of Arbekacin Sulfate taken

Internal standard solution—A solution of bekanamycin sulfate (1 in 2000)

Operating conditions

Detector: A spectrofluorometer (excitation wavelength: 340 nm, fluorescence wavelength: 460 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Reaction coil: A column about 0.3 mm in internal diameter and about 3 m in length

Reaction coil temperature: A constant temperature of about 50 °C

Mobile phase: Dissolve 8.70 g of sodium 1-pentanesulfonate and 8.52 g of anhydrous sodium sulfate in 980 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 230 mL of this solution add 20 mL of methanol.

Reaction reagent: Dissolve 12.36 g of boric acid in 960 mL of water, add a solution obtained by dissolving 0.4 g of *o*-phthaldehyde in 10 mL of ethanol (99.5), adjust the pH to 10.5 with 8. mol/L potassium hydroxide TS, and add water to make 1000 mL. To this solution add 1 mL of 2-mercaptoethanol.

Reaction temperature: A constant temperature of about 50 °C

Flow rate of mobile phase: 0.5 mL/minute

Flow rate of reaction reagent: 1 mL/minute

System suitability

System performance: Dissolve 20 mg each of Arbekacin Sulfate, bekanamycin sulfate, and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, bekanamycin, arbekacin, and dibekacin are eluted in this order with the resolutions between the peaks of bekanamycin and arbekacin and between the peaks of arbekacin and dibekacin being not less than 5 and not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dibekacin to that of the internal standard is not more than 2.0 %.

(4) ***Related substances***—Dissolve 20 mg of Arbekacin Sulfate in 20 mL of water, and use this solution as the test solution. Pipet 3 mL of the test solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the total area of the peaks other than arbekacin and dibekacin from the test solution is not larger than the peak area of arbekacin from the standard solution.

Operating conditions

Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Purity (3).

System suitability

System performance: Dissolve 10 mg each of Arbekacin Sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, arbekacin and dibekacin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of arbekacin is not more than 5.0 %.

Time span of measurement: About 1.5 times as long as the retention time of arbekacin

Loss on Drying Not more than 5.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement, when Arbekacin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of arbekacin, when Arbekacin Sulfate is used in a sterile preparation.

Assay ***The Cyliner-plate method*** (1) Test organism- *Bacillus subtilis* ATCC 6633

(2) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be between 7.8 and 8.0 after sterilization.

(3) Standard solution- Weigh accurately about 20 mg (potency) of Arbekacin Sulfate RS, previously dried, dissolve in diluted phosphate buffer solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15 °C and use within 30 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(4) Test solution – Weigh accurately an amount of Arbekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Arbekacin Sulfate Injection

Arbekacin Sulfate Injection is an aqueous injection. Arbekacin Sulfate Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of arbekacin ($C_{22}H_{44}N_6O_{10}$: 552.62).

Method of Preparation Prepare as directed under Injections, with Arbekacin Sulfate.

Description Arbekacin Sulfate Injection appears as a clear and colorless liquid.

Identification To 0.2 mL of Arbekacin Sulfate Injection add 1 mL of water, and use this solution as the test solution. Separately, dissolve 10 mg of Arbekacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform, and ethanol (95) (7 : 6 : 4 : 1) to a distance ofa bout 12 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate and heat at 80 °C for 10 minutes: the principal spot obtained from the test solution and the spot from the standard solution are purple-brown in color and have the same R_f value.

Osmotic Pressure Ratio 0.8 ~ 1.2 (for the preparation intended for intramuscular use)

pH 6.0 ~ 8.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of arbekacin.

Foreign Insoluble Matter Test It meets the requirement.

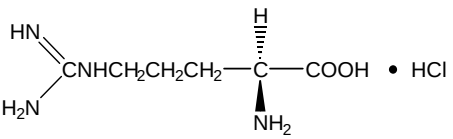
Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Proceed as directed in the Assay under Arbekacin Sulfate. Pipet an amount of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency) of arbekacin sulfate according to the labeled amount, and add water to make exactly 50 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers.

L-Arginine Hydrochloride



L-Arginine Hydrochloride $C_6H_{14}N_4O_2 \cdot HCl$: 210.66

Arginine hydrochloride [*1119-34-2*]

L-Arginine Hydrochloride, when dried, contains not

less than 98.5 % and not more than 101.0 % of L-arginine hydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$).

Description L-Arginine Hydrochloride appears as white crystals or crystalline powder, is odorless and has a slight, characteristic taste.
L-Arginine Hydrochloride is freely soluble in water or in formic acid, very slightly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) Determine the infrared spectra of L-Arginine Hydrochloride and L-Arginine Hydrochlorode RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of L-Arginine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the pH of this solution is between 4.7 and 6.2.

Specific Optical Rotation $[\alpha]_D^{20}$: +21.5 ~ +23.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.6 g of L-Arginine Hydrochloride and prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(3) ***Ammonium***—Perform the test with 0.25 g of L-Arginine Hydrochloride, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(4) ***Heavy metals***—Proceed with 1.0 g of L-Arginine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of L-Arginine Hydrochloride according to Method 1 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.20 g of L-Arginine Hydrochloride in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution and add water to make exactly 10 mL. Pipet 1 mL of this solution and add water to make exactly 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water, 1-butanol and ammonia solution (28) (2 : 1 : 1 : 1) to a distance of about 10 cm and dry the plate at 100 ºC for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 ºC for 5 minutes: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, 105 ºC, 3 hours).

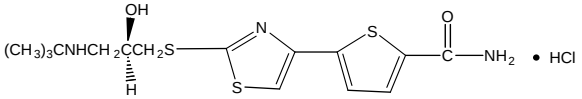
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of L-Arginine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS and heat in a water-bath for 30 minutes. After cooling, add 45 mL of acetic acid (100) and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.533 mg of $C_6H_{14}N_4O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Arotinolol Hydrochloride



and enantiomer

$C_{15}H_{21}N_3O_2S_3 \cdot HCl$: 408.00

5-[2-[3-(*tert*-Butylamino)-2-hydroxypropyl]sulfanyl-1,3-thiazol-4-yl]thiophene-2-carboxamide hydrochloride [*68377-91-3*]

Arotinolol Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of arotinolol hydrochloride ($C_{15}H_{21}N_3O_2S_3 \cdot HCl$).

Description Arotinolol Hydrochloride is a white to pale yellow crystalline powder.
Arotinolol Hydrochloride is freely soluble in dimethylsulfoxide, slightly soluble in methanol or in water, very slightly soluble in ethanol (99.5), and practically insoluble in ether.
A solution of Arotinolol Hydrochloride in methanol (1 in 125) shows no optical rotation.

Identification (1) Determine absorption spectra of solutions of Arotinolol Hydrochloride and arotinolol hydrochloride RS, in methanol (1 in 75000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Arotinolol

Hydrochloride and Arotinolol Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The solution of Arotinolol Hydrochloride (1 in 200) responds to Qualitative Tests (2) for Chloride.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Arotinolol Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 50 mg of Arotinolol Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 40 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (30 : 10 : 10 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 g (1 g, in vacuum, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

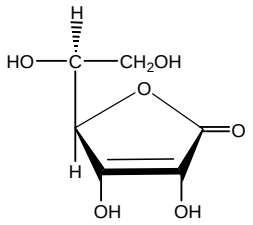
Assay Weigh accurately about 1.5 g of Arotinolol Hydrochloride, previously dried and dissolve in dimethylsulfoxide to make exactly 25 mL. Pipet 5 mL of this solution, add 100 mL of water and 5 mL of sodium hydroxide TS and extract with three 50 mL volumes of dichloromethane. Filter each dichloromethane extract through a pledget of absorbent cotton with anhydrous sodium sulfate on it. Evaporate combined filtrate to dryness in vacuum. Dissolve the residue in 70 mL of acetic acid (100) and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 20.400 mg of $C_{15}H_{21}N_3O_2S_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ascorbic Acid



Vitamin C $C_6H_8O_6$: 176.12

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxy-2,5-dihydrofuran-2-one [*50-81-7*]

Ascorbic Acid, when dried, contains not less than 99.0 % and not more than 101.0 % of ascorbic acid ($C_6H_8O_6$).

Description Ascorbic Acid appears as white crystals or a white crystalline powder, is odorless and has an acid taste.

Ascorbic Acid is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in ether.

Melting point—About 190 ºC (with decomposition)

Identification (1) To 5 mL each of solution of Ascorbic Acid (1 in 50), add 1 drop of potassium permanganate TS or 1 to 2 drops of 2,6-dichlorophenolindophenol sodium TS: the color of the solution is discharged immediately in each case .

(2) Dissolve 0.1 g of Ascorbic Acid in 100 mL of a solution of metaphosphoric acid (1 in 50). To 5 mL of this solution, add iodine TS until the color of the solution becomes pale yellow. Then add 1 drop of a solution of cupric sulfate (1 in 1000) and 1 drop of pyrrole and warm the mixture at 50 ºC for 5 minutes: a blue color develops.

Specific Optical Rotation $[\alpha]_D^{20}$: +20.5 ~ +21.5° (2.5 g, water, 25 mL, 100 mm).

pH Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the pH of this solution is between 2.2 and 2.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 of Ascorbic Acid in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—To 2.0 g of Ascorbic Acid add water to make 20 mL. Take 12 mL of this solution and use as the test solution. Separately, to 10 mL of diluted standard lead solution add 2 mL of the test solution and use this solution as the control solution. To 10 mL of water add 2 mL of the test solution and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not

more intense than that of the control solution (not more than 10 ppm).

Diluted standard lead solution—Immediately before use, pipet 5 mL of the standard lead solution and add water to make 50 mL.

System suitability: The control solution shows a pale brown color compared to the blank solution.

(3) ***Copper***—Dissolve 2.0 g of Ascorbic Acid in 2 mL of 0.1 mol/L nitric acid, and add 25.0 mL of 0.1 mol/L nitric acid. Pipet a suitable amount of the standard copper solution, add 0.1 mol/L nitric acid to make 0.2, 0.4, and 0.6 ppm, and use these solutions as standard solutions (1), (2), and (3), respectively. Perform the test with the test solution and standard solutions (1), (2), and (3) as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the content of copper in the test solution using the calibration curve obtained from the standard solutions (not more than 5 ppm). Use 0.1 mol/L nitric acid as the blank solution.

Gas: Dissolved acetylene – Air
Lamp: Copper hollow cathode lamp
Wavelength: 324.8 nm

Loss on Drying Not more than 0.2 % (1 g, silica gel, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Ascorbic Acid, previously dried, dissolve in 50 mL of a solution of meta-phosphoric acid (1 in 50) and titrate with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 8.806 mg of $C_6H_8O_6$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Ascorbic Acid Injection

Vitamin C Injection

Ascorbic Acid Injection is an aqueous solution for injection. Ascorbic Acid Injection contains not less than 95.0 % and not more than 115.0 % of the labeled amount of ascorbic acid ($C_6H_8O_6$: 176.12).

Method of Preparation Prepare as directed under Injections, with the sodium salt of ascorbic acid.

Description Ascorbic Acid Injection is a clear, colorless liquid.

Identification (1) Measure a volume of Ascorbic Acid Injection, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, and add water to make 25 mL. Proceed with 5 mL each of the solution as directed in the Identification (1) under Ascorbic Acid.

(2) Measure a volume of Ascorbic Acid Injection, equivalent to 5 mg of Ascorbic Acid according to the labeled amount. Add a solution of metaphosphoric acid (1 in 50) to make 5 mL and proceed with this solution as directed in the Identification (2) under Ascorbic Acid.

(3) Ascorbic Acid Injection responds to the Qualitative Tests (1) for sodium salt.

pH 5.6 ~ 7.4.

Purity ***Oxalate***—Measure a volume of Ascorbic Acid Injection, equivalent to 50 mg of ascorbic acid, and add water to make 5 mL. Add 0.2 mL of acetic acid (31) and 0.5 mL of calcium chloride TS, and allow to stand for 1 minute: no turbidity is produced.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.15 EU/mg of ascorbic acid.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Ascorbic Acid Injection, equivalent to about 0.1 g of ascorbic acid ($C_6H_8O_6$), previously diluted with metaphosphoric acid-acetic acid TS, if necessary and add metaphosphoric acid-acetic acid TS to make exactly 200 mL. Pipet 2 mL of this solution and proceed with this solution as directed in the Assay under Ascorbic Acid Powder.

Each mL of 2,6-dichlorophenol-indophenol sodium TS for titration = A mg of $C_6H_8O_6$

Containers and Storage ***Containers***—Hermetic containers.
Storage—Under nitrogen atmosphere.

Ascorbic Acid Powder

Vitamin C Powder

Ascorbic Acid Powder contains not less than 95.0 % and not more than 120.0 % of the labeled amount of

ascorbic acid ($C_6H_8O_6$: 176.12).

Method of Preparation Prepare as directed under Powders, with Ascorbic Acid.

Identification (1) Weigh a portion of Ascorbic Acid Powder, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, add 30 mL of water, shake for 1 minute and filter. Proceed with 5 mL each of the filtrate as directed in the Identification (1) under Ascorbic Acid.

(2) Weigh a portion of Ascorbic Acid Powder, equivalent to about 10 mg of Ascorbic Acid according to the labeled amount, add 10 mL of a solution of metaphosphoric acid (1 in 50), shake for 1 minute and filter. Proceed with 5 mL of the filtrate as directed in the Identification (2) under Ascorbic Acid.

Purity ***Rancidity***—Ascorbic Acid Powder is free from any unpleasant or rancid odor and taste.

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately a portion of Ascorbic Acid Powder, equivalent to about 0.1 g of ascorbic acid ($C_6H_8O_6$) according to the labeled amount, extract with several successive portions of metaphosphoric acid-acetic acid TS, combine the extracts and filter. Wash the residue with metaphosphoric acid-acetic acid TS. Combine the filtrate and washings and add metaphosphoric acid-acetic acid to make exactly 200 mL. Pipet 2 mL of this solution and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate with 2,6-dichlorophenol-indophenol sodium TS for titration until a pale red color persists for 5 seconds. Perform a blank determination and make any necessary correction.

Each mL of 2,6-dichlorophenol-indophenol sodium TS for titration = *A* mg of $C_6H_8O_6$

A is decided by the following standardization of 2,6-dichlorophenol-indophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium TS for titration. Preparation—Dissolve 42 mg of sodium bicarbonate in 50 mL of water, add 50 mg of 2,6-dichlorophenol-indophenol sodium and water to make 200 mL and filter. Prepare before use.

Standardization—Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS and titrate with 2,6-dichlorophenol-indophenol sodium until a pale red color persists for 5 seconds. Perform a blank determination and make any necessary correction. Calculate the quantity (*A* mg) of ascorbic acid ($C_6H_8O_6$) equivalent to 1 mL of this test solution.

Containers and Storage ***Containers***—Tight containers.

Ascorbic Acid Tablets

Vitamin C Tablets

Ascorbic Acid Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of ascorbic acid ($C_6H_8O_6$: 176.12).

Method of Preparation Prepare as directed under Tablets, with Ascorbic Acid.

Identification (1) Weigh a portion of powdered Ascorbic Acid Tablets, equivalent to 0.5 g of Ascorbic Acid according to labeled amount, add 30 mL of water, shake for 1 minute and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Ascorbic Acid.

(2) Pipet 2 mL of the filtrate, add sodium hydroxide TS to make neutral, then add 2 drops of uranyl acetate TS: a red brown color develops. And then add 2 mL of sodium hydroxide TS: a yellow color develops.

(3) Weigh a portion of powdered Ascorbic Acid Tablets, equivalent to 10 mg of Ascorbic Acid according to labeled amount, add 10 mL of a solution of metaphosphoric acid (1 in 50), shake for 1 minute and filter. Proceed with 5 mL of the filtrate as directed in the Identification (2) under Ascorbic Acid.

Dissolution Test Perform the test with 1 tablet of Ascorbic Acid Tablets at 50 revolutions per minute according to the Method 2 under the Dissolution Test, using 900 mL of the water as the dissolution solution. After 45 minutes from the start of the test, take not less than 20 mL of the dissolution solution and filter the solution using a membrane filter having the pore size of not more than 0.8 μm. Proceed with the filtrate as directed in the Assay.
The dissolution rate of Ascorbic Acid Tablets in 45 minutes is not less than 75 %.

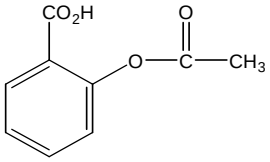
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Ascorbic Acid Tablets, equivalent to about 0.1 g of ascorbic acid ($C_6H_8O_6$) and proceed with this powder as directed in the Assay under Ascorbic Acid Powder.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Aspirin



Acetylsalicylic Acid $C_9H_8O_4$: 180.16

2-Acetyloxybenzoic acid [*50-78-2*]

Aspirin, when dried, contains not less than 99.5 % and not more than 101.0 % of aspirin ($C_9H_8O_4$).

Description Aspirin appears as white crystals, granule or powder, is odorless and has a slight acid taste.
Aspirin is freely soluble in ethanol (95) or in acetone, soluble in ether, and slightly soluble in water.
Aspirin dissolves in sodium hydroxide TS and in sodium carbonate TS.
In moist air, aspirin gradually hydrolyzes to salicylic acid and acetic acid.

Melting point—About 136 ºC (bath fluid is heated at 130 ºC previously).

Identification (1) Boil 0.1 g of Aspirin in 5 mL of water for 5 to 6 minutes, cool. and add 1 to 2 drops of iron (III) chloride TS: a red-purple color is produced.

(2) Boil 0.5 g of Aspirin in 10 mL of sodium carbonate TS for 5 minutes and add 10 mL of dilute sulfuric acid: the odor of acetic acid is perceptible and a white precipitate is produced. Filter the precipitate, add 3 mL of ethanol (95) and 3 mL of sulfuric acid to the filtrate and heat: the odor of ethyl acetate is perceptible.

Purity (1) ***Clarity of solution***—Dissolve 0.5 g of Aspirin in 10 mL of warm sodium carbonate TS: the solution is clear.

(2) ***Salicylic acid***—Dissolve 2.5 g of Aspirin in ethanol (95) to make 25 mL and add 1.0 mL of this solution to a solution prepared by transferring 1 mL of a freshly prepared dilute ammonium iron (III) sulfate TS to a Nessler tube and diluting with water to make 50 mL. Allow to stand for 30 seconds: the color of the solution is not more intense than that of the following control solution.

Control solution—Dissolve 0.1 g of salicylic acid in water and add 1 mL of acetic acid (100) and water to make 1000 mL. Add 1.0 mL of this solution to a solution prepared by transferring 1 mL of freshly prepared dilute ammonium iron (III) sulfate TS and 1 mL of ethanol (95) to a Nessler tube and diluting with water to make 50 mL. Allow to stand for 30 seconds.

(3) ***Chloride***—Boil 1.8 g of Aspirin in 75 mL of water for 5 minutes, cool, add water to make 75 mL and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015 %).

(4) ***Sulfate***—To 25 mL of the filtrate obtained in (3), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040 %).

(5) ***Heavy metals***—Dissolve 2.5 g of Aspirin in 30 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of standard lead solution, 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) ***Readily carbonizable substances***—Weigh 0.5 g of Aspirin and perform the test. The solution has no more color than Color Matching Fluid Q.

Loss on Drying Not more than 0.5 % (3 g, silica gel, 5 hours).

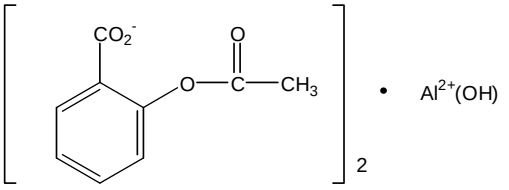
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1.5 g of Aspirin, previously dried, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and boil gently for 10 minutes using a reflux condenser closed with a carbon dioxide-absorbing tube (soda lime). Cool and titrate excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS) immediately. Perform a blank determination and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 45.04 mg of $C_9H_8O_4$

Containers and Storage ***Containers***—Well-closed containers.

Aspirin Aluminum



Aluminum Acetyl Salicylate $C_{18}H_{15}AlO_9$: 402.29

Aluminum 2-acetyloxybenzoate hydroxide
[*23413-80-1*]

Aspirin Aluminum contains not less than 83.0 % and not more than 90.0 % of aspirin ($C_9H_8O_4$: 180.16) and not less than 6.0 % and not more than 7.0 % of aluminum (Al: 26.98), calculated on the anhydrous basis.

Description Aspirin Aluminum is a white, crystalline powder. Aspirin Aluminum is odorless or has a slightly acetic odor.
Aspirin Aluminum is practically insoluble in water, in methanol, in ethanol (95) or in ether.
Aspirin Aluminum dissolves, with decomposition, in sodium hydroxide TS or sodium carbonate TS.

Identification (1) Dissolve 0.1 g of Aspirin Aluminum in 10 mL of sodium hydroxide TS by heating, if necessary. Neutralize 2 mL of this solution with hydrochloric acid and add 1 to 2 drops of iron (III) chloride TS: a red-purple color develops.

(2) Determine the absorption spectrum of the test solution obtained in the Assay (1) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 277 nm and 279 nm.

(3) Place 2 g of Aspirin Aluminum in a platinum crucible and ignite until charred. To the residue, add 1 g of anhydrous sodium carbonate and ignite for 20 minutes. After cooling, to the residue, add 15 mL of dilute hydrochloric acid, shake and filter: the filtrate responds to the Qualitative Tests for aluminum salt.

Purity (1) ***Salicylate***—Using A_{T2} and A_{S2} obtained in the Assay (1), calculate the amount of salicylate [as salicylic acid ($C_7H_6O_3$: 138.12)] by the following equation: salicylate content is not more than 7.5 %, calculated on the anhydrous basis.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = \text{Amount (mg) of Salicylic Acid RS} \times \frac{A_{T2}}{A_{S2}} \times \frac{1}{4}$$

(2) ***Heavy metals***—Place 2.0 g of Aspirin Aluminum in a porcelain crucible, cover the crucible loosely and ignite at a low temperature until charred. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid to the content of the crucible, heat gently the crucible until white fumes are evolved and continue the heating until white fumes are no longer evolved, then ignite between 500 ºC and 600 ºC until the carbon is incinerated. When the incineration is not completed, add 2 mL of nitric acid and 1 mL of sulfuric acid and heat gently in the same manner, then ignite between 500 ºC and 600 ºC to incinerate completely. After cooling, add 2 mL of hydrochloric acid and proceed as directed in Method 2 and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution and add 2.0 mL of standard lead solution and water to make 50 mL (not more than 10 ppm).

(3) ***Arsenic***—Dissolve 1.0 g of Aspirin Aluminum in 15 mL of sodium hydroxide TS. To this solution, add 1 drop of phenolphthalein TS and with stirring, add drop-wise hydrochloric acid until the red color of the solution disappears. Then add 2 mL of hydrochloric acid, cool with occasional shaking for 10 minutes and filter with a glass filter (G3). Wash the residue with two 5 mL volumes of 1 mol/L hydrochloric acid TS and combine the filtrate and the washings. Use this solution as the test solution and perform the test (not more than 2 ppm).

Water Not more than 4.0 % (0.15 g, volumetric titration, direct titration).

Assay (1) ***Aspirin***—Weigh accurately about 0.1 g of Aspirin Aluminum, add 40 mL of sodium fluoride TS and shake for 5 minutes. Allow the solution to stand for 10 minutes with frequent shaking. Extract the solution with six 20 mL volumes of chloroform. Combine all chloroform extracts and add chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 90 mg of Salicylic Acid RS, previously dried in a desiccator (silica gel) for 3 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 5 mL of this solution, add chloroform to make exactly 200 mL and use this solution as the standard solution (1). Then weigh accurately about 90 mg of Aspirin RS, previously dried in a desiccator (silica gel) for 5 hours and dissolve in chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Ultraviolet-visible Spectrophotometry. Determine the absorbances, A_{T1} and A_{S1}, of the test solution and the standard solution (1) at 278 nm and absorbances, A_{T2} and A_{S2}, of the test solution and the standard solution (2), respectively, at 308 nm. Then determine the absorbance, A_{S3} of the standard solution (2) at 278 nm.

$$\text{Amount (mg) of aspirin } (C_9H_8O_4) = \text{Amount (mg) of Aspirin RS} \times \frac{A_{T1} - \dfrac{A_{T2} \times A_{S1}}{A_{S2}}}{A_{S3}}$$

(2) ***Aluminum***—Weigh accurately about 0.4 g of Aspirin Aluminum and dissolve in 10 mL of sodium hydroxide TS. Add drop-wise 1 mol/L hydrochloric acid TS until the pH of the solution to about 1, add 20 mL of acetic acid-ammonium acetate buffer solution, pH 3.0 and 0.5 mL of Cu-PAN TS and heat. While boiling, titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red to yellow and persists for 1 minute. Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraahetate VS
= 1.3491 mg of Al

Containers and Storage ***Containers***—Well-closed containers.

Aspirin Tablets

Acetylsalicylic Acid Tablets

Aspirin Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of aspirin ($C_9H_8O_4$: 180.16).

Method of Preparation Prepare as directed under Tablets, with Aspirin.

Identification (1) Weigh a portion of powdered Aspirin Tablets, equivalent to 0.1 g of Aspirin according to the labeled amount, add 10 mL of water and boil for 5 to 6 minutes. After cooling, filter and add 1 to 2 drops of iron (III) chloride TS to the filtrate: a red-purple color develops.

(2) Weigh a portion of powdered Aspirin Tablets, equivalent to 0.5 g of Aspirin according to the labeled amount, extract with two 10 mL volumes of warm ethanol and filter the combined extracts. Evaporate the filtrate to dryness and boil the residue with 10 mL of sodium carbonate TS for 5 minutes. Proceed as directed in the Identification (2) under Aspirin.

Purity ***Salicylic acid***—Weigh accurately and powder not less than 20 Aspirin Tablets. Weigh accurately an amount equivalent to about 1.0 g of Aspirin, add mobile phase to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 15 mg of Salicylic Acid RS, dissolve in mobile phase to make 100 mL. Pipet 5 mL of this solution, add mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with test solution and the standard solution as directed under Assay (not more than 0.15 %).

Disintegration Test It meets the requirement..

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Aspirin Tablets. Weigh accurately an amount equivalent to about 0.25 g of aspirin ($C_9H_8O_4$), add mobile phase, shake well, add mobile phase to make 200 mL, then filter. Discard the first 10 mL of the filtrate, measure exactly 2 mL of subsequent filtrate, add 4.0 mL of internal standard solution and mobile phase to make 100 mL. Use this solution as the test solution. Separately, weigh accurately about 25 mg of Aspirin RS, previously dried in desiccator (silica gel) for 5 hours, dissolve in mobile phase to make exactly 20 mL. Pipet 2 mL of this solution, add 4.0 mL of internal standard solution and mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of aspirin to that of internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of aspirin } (C_9H_8O_4) = \text{Amount (mg) of Aspirin RS} \times \frac{Q_T}{Q_S} \times 10$$

Internal standard solution—Dissolve about 10 mg of Theophylline RS in mobile phase to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and between 15 and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (between 5 and 10 μm in particle diameter).

Column temperature: A constant temperature of about 30 ºC.

Mobile phase: A mixture of 0.085 % phosphoric acid and methanol (60 : 40).

Flow rate: 1.0 mL/minute.

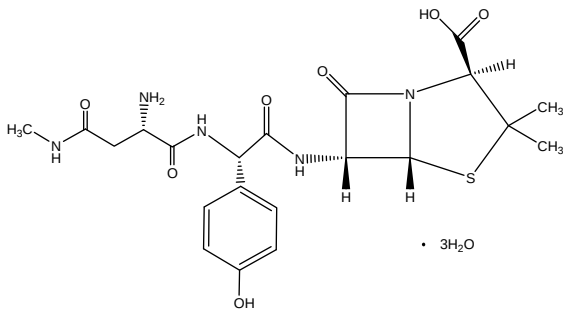
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and aspirin are eluted in this order with the resolution between their peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Aspoxicillin Hydrate



Aspoxicillin $C_{21}H_{27}N_5O_7S \cdot 3H_2O$

(3*S*,5*R*,6*R*)-6-{(2*R*)-2-[(2*R*)-2-Amino-4-(methylamino)-4-oxobutanamido]-2-(4-hydroxyphenyl)acetamido}-2,2-dimethylpenam-3-carboxylic acid trihydrate [*117774-38-6*]

Aspoxicillin Hydrate contains not less than 950 μg (potency) and 1020 μg (potency) per mg of aspoxicillin ($C_{21}H_{27}N_5O_7S$: 493.53), calculated on the anhydrous basis.

Description Aspoxicillin Hydrate appears as white crystals or crystalline powder.
Aspoxicillin Hydrate is freely soluble in *N,N*-dimethylformamide, sparingly soluble in water, and practically insoluble in methanol, in ethanol (95), or in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Aspoxicillin Hydrate and Aspoxicillin RS (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Aspoxicillin Hydrate and Aspoxicillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +170 ~ +185° (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g (potency) of Aspoxicillin Hydrate in 50 mL of water is between 4.2 and 5.2.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Aspoxicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Aspoxicillin Hydrate according to Method 5, and perform the test (not more than 1 ppm).

(4) ***Related substances***—Dissolve 0.05 g of Aspoxicillin Hydrate in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of each peak other than aspoxicillin from the test solution is not larger than 3/10 times the peak area of aspoxicillin from the standard solution, and the total area of the peaks other than aspoxicillin from the test solution is not larger than the peak area of aspoxicillin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aspoxicillin obtained from 10 μL of this solution is equivalent to 15 to 25 % of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aspoxicillin is not more than 5 %.

Time span of measurement: About 6 times as long as the retention time of aspoxicillin.

Water 9.5 ~ 13.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Aspoxicillin Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of aspoxicillin, when Aspoxicillin Hydrate is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Aspoxicillin Hydrate and Aspoxicillin RS, dissolve each in a suitable amount of water, add exactly 10 mL of the internal standard solution, 6.5 mL of acetonitrile, and water to make 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aspoxicillin to that of the internal standard.

$$\text{Amount [μg (potency)] of aspoxicillin } (C_{21}H_{27}N_5O_7S) = \text{Amount [μg (potency)] of Aspoxicillin} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *N*-(3-hydroxyphenyl)acetamide (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: To 130 mL of acetonitrile add potassium dihydrogen phosphate TS (pH 3.0) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of aspoxicillin is about 3 minutes.

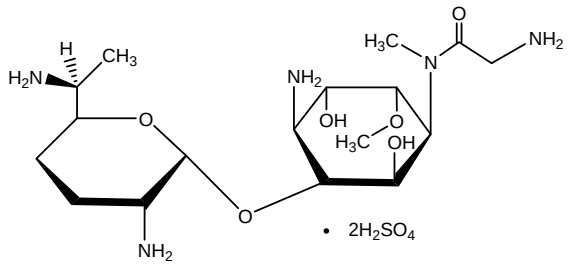
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspoxicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deivation of the ratios of the peak area of aspoxicillin to that of the internal standard is not more than 0.8 %.

Containers and Storage ***Containers***—Tight containers.

Astromicin Sulfate



$C_{17}H_{35}N_5O_6 \cdot 2H_2SO_4$: 601.65

2-Amino-*N*-[(1*S*,2*R*,3*R*,4*S*,5*S*,6*R*)-4-amino-3-[(2*R*,3*R*,6*S*)-3-amino-6-(1-aminoethyl)oxan-2-yl]oxy-2,5-dihydroxy-6-methoxycyclohexyl]-*N*-methylacetamide disulfate [*72275-67-3*]

Astromicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora olivasterospora*.
Astromicin Sulfate contains not less than 610 μg (potency) and not more than 680 μg (potency) per mg of astromicin ($C_{17}H_{35}N_5O_6$: 405.49), calculated on the anhydrous basis.

Description Astromicin Sulfate appears as white to pale yellowish white powder or masses.
Astromicin Sulfate is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol or in ethanol (99.5).
Astromicin Sulfate is hygroscopic.

Identification (1) To 2 mL of a solution of Astromicin Sulfate (1 in 100) add 2 to 3 drops of barium chloride TS: a white precipitate is produced, and it does not dissolve by addition of dilute nitric acid.

(2) Dissolve separately 10 mg each of Astromicin Sulfate and Astromicin Sulfate in 10 mL of water. To 5 mL each of these solutions add water to make 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the retention time of the peak of astromicin obtained from the test solution corresponds to that from the standard solution.

Operating conditions

Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Purity (3).

Specific Optical Rotation $[\alpha]_D^{20}$: +90 ~ +110° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Astromicin Suflate in 10 mL of water is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Astromicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Astromicin Suflate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Astromicin Sulfate in 100 mL of water, and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the peak areas of the related substances, having the relative retention times of about 0.1 and about 1.2 with respect to the peak of astromicin, are not larger than the peak area of astromicin from the standard solution, and the peak area of the related substance, having the relative retention time of about 0.8, is not larger than 2.0 times the peak area of astromicin from the standard solution. The total area of the peaks other than astromicin is not larger than 3.5 times the peak area of astromicin from the standard solution.

Operating conditions

Detector: A spectrofluorometer (excitation wavelength: 340 nm, fluorescence wavelength: 430 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Reaction coil: A stainless steel column 0.25 mm in internal diameter and 150 cm in length

Reaction coil temperature: 50 °C

Mobile phase: Dissolve 25 mL of a solution of sodium 1-heptanesulfonate (1 in 1000) and 1 mL of acetic acid (100) in 800 mL of a solution of anhydrous sodium sulfate (71 in 2000), and add water to make 1000 mL.

Reaction reagent: Dissolve 11.2 g of potassium hydroxide, 0.458 g of polyoxyethylene (23) lauryl ether, 0.300 g of *o*-phthaldehyde, and 1 mL of 2-mercaptoethanol in 400 mL of a solution of boric acid (31 in 1000), and add water to make 500 mL.

Reaction temperature: 50 °C

Flow rate of mobile phase: 0.7 mL/minute

Flow rate of reaction reagent: 0.2 mL/minute

System suitability

Test for required detectability: To 5 mL of the test solution add water to make 100 mL, and use this solution as the system suitability solution. Pipet 2 mL of this solution, and add water to make exactly 100 mL. Confirm that the peak area of astromicin obtained from 10 μL of this solution is equivalent to 1.5 to 2.5 % of that from the system suitability solution.

System performance: To 5 mL of the test solution and 2 mL of a solution of L-valine (1 in 5000) add 100 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, L-valine and astromicin are eluted in this order with the resolution between these peaks being not less than 1.5. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the symmetry factor of the peak of astromicin is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of astromicin is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of astromicin.

Water Not more than 8.0 % (0.2 g, volumetric titration, back titration. Use a mixture of methanol for water determination and ethylene glycol for water determination (1 : 1) instead of methanol for water determination.)

Sterility Test It meets the requirement, when Astromicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of astromicin, when Astromicin Sulfate is used in a sterile preparation.

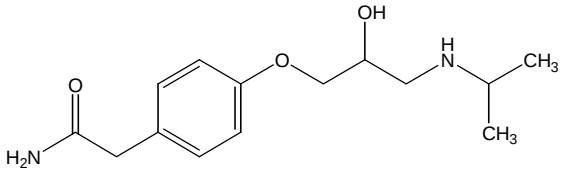
Assay ***The Cylinder-plate method*** (1) Test organism- *Bacillus subtilis* ATCC 6633

(2) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(3) Weigh accurately about 25 mg (potency) of Astromicin Sulfate, and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 25 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 25 mg (potency) of Astromicin Sulfate RS, dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15 °C, and use within 30 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Atenolol



and enantiomer

$C_{14}H_{22}N_2O_3$: 266.34

2-[4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide [*29122-68-7*]

Atenolol, when dried, contains not less than 99.0 % and not more than 101.0 % of atenolol ($C_{14}H_{22}N_2O_3$).

Description Atenolol is a white to pale yellow crystalline powder.

Atenolol is freely soluble in methanol or in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.

A solution of Atenolol in methanol (1 in 25) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Atenolol and Atenolol RS, in methanol (5 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths..

(2) Determine the infrared spectra of Atenolol and Atenolol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 152 ~ 156 ºC.

Purity (1) ***Chloride***—Dissolve 1.0 g of Atenolol in 100 mL of 0.15 mol/L nitric acid solution, and use this solution as the test solution. Dissolve 1.4 mL of 0.02 mol/L hydrochloric acid TS in 100 mL of 0.15 mol/L nitric acid solution, and use this solution as the control solution. To the test solution and control solution add 1 mL of silver nitrate TS and allow to stand for 5 mintutes: the test solution is not more turbid than the control solution (not more than 0.1 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Atenolol according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of Standard lead solution (not more than 20 ppm).

(3) ***Related substances***— Dissolve 50 mg of Atenolol in the mobile phase to make 25 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: any area peak other than atenolol obtained with the test solution is not larger than 1/2 times the peak area of atenolol from the standard solution, and the total area of all peaks other than atenolol from the test solution is not larger than the peak area of atenolol with the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 226 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 3.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 40 volume of this solution add 9 volume of methanol and 1 volume of tetrahydrofuran. Dissolve 1.0 g of sodium 1-octanesulfonate and 0.4 g of tetrabutylammonium hydrogensulfate in 1000 mL of this solution.

Flow rate: Adjust the flow rate so that the retention time of atenolol is about 8 minutes.

System suitability

Test for required detectability: To exactly 10 mL of the standard solution add mobile phase to make exactly 50 mL. Confirm that the peak area of atenolol obtained with 10 μL of this solution is equivalent to 14 to 26 % of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atenolol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atenolol is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of atenolol.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hour).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g each of Atenolol and Atenolol RS, previously dried, dissolve separately in the mobile phase to make exactly 100 mL, respectively. Pipet 5 mL each of these solutions, add the mobile phase to make exactly 50 mL, pipet 5 mL each of these solutions again, add the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate each peak area of atenolol, A_T and A_S, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of atenolol } (C_{14}H_{22}N_2O_3) = \text{Amount (mg) of Atenolol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 226 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous sodium dihydrogen phosphate in 700 mL of water, add 2 mL of dibutylamine, and adjust the pH to 3.0 with 0.8 mol/L phosphoric acid TS. Add 300 mL of methanol to this solution and mix well.

Flow rate: 1.7 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Atenolol Tablets

Atenolol tablets contains not less than 90.0 % and not more than 110.0 % of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$: 266.34).

Method of Preparation Prepare as directed under Tablets, with Atenolol.

Identification (1) To a portion of powdered Atenolol Tablets, equivalent to 0.1 g of Atenolol according to labeled amount, add 15 mL of methanol, mix well, warm at 50 ºC, shake for 5 minutes. Filter, evaporate the filtrate on a water-bath to dryness, add 0.1 mol/L hydrochloric acid to this residue, shake on warming, and filter. To this filtrate, add a sufficient amount of 1 mol/L sodium hydroxide TS to make alkaline, extract with 10 mL of chloroform, remove water from the chloroform layer using anhydrous sodium sulfate, and filter. Evaporate the filtrate to dryness on a water-bath, dry this residue at 105 ºC for 1 hour, and use this residue as the test sample. Determine the infrared spectra of the test sample and Atenolol RS, powdered finely according to the same method, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of main peak of the test solution for Assay and the standard solution is the same.

Dissolution Test Perform the test with 1 tablet of Atenolol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL or more of the dissolve solution 30 minutes after starting the test, and filter through a membrane filter with a pore size of not more than 0.8 μm. Add phosphoric acid solution (1 in 1000) to this filtrate so that each mL contains 10 μg of atenolol ($C_{14}H_{22}N_2O_3$), and use this solution as the test solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, prepared according to the Assay, respectively, as directed in the Assay.

The dissolution rate of Atenolol Tablets in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of Atenolol ($C_{14}H_{22}N_2O_3$) = $900 \times C \times D \times \frac{A_T}{A_S}$

C: Concentration of Atenolol in the standard solution (mg/mL).

D: Dilution times of the test solution.

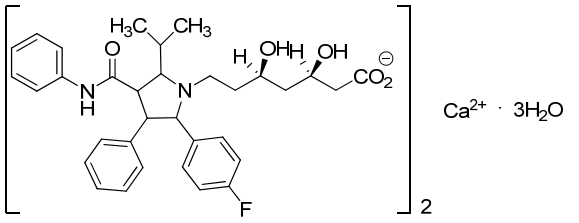
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Atenolol Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of atenolol ($C_{14}H_{22}N_2O_3$), add the mobile phase, and sonicate for 15 minutes. Add the mobile phase to make exactly 1000 mL, centrifuge, pipet 2 mL of the clear supernatant liquid, add the mobile phase to make 50 mL, and use this solution as the test solution. Separately, weigh exactly about 25 mg of Atenolol RS, add the mobile phase to make 100 mL, pipet 2 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed in the Assay under Atenolol.

$$\text{Amount (mg) of atenolol } (C_{14}H_{22}N_2O_3) = \text{Amount (mg) of Atenolol RS} \times \frac{A_T}{A_S} \times 10$$

Containers and Storage ***Containers***—Tight containers.

Atorvastatin Calcium Hydrate



$C_{66}H_{68}CaF_2N_4O_{10}\cdot 3H_2O$: 1209.42

Calcium *bis*{(3*R*,5*R*)-7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate} trihydrate [*344423-98-9*]

Atorvastatin Calcium Hydrate contains not less than 98.0 % and not more than 102.0 % of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$), calculated on the anhydrous basis.

Description Atorvastatin Calcium Hydrate appears as white to pale yellow crystalline powder.

Atorvastatin Calcium is very soluble in methanol, freely soluble in dimethylsulfoxide and very slightly soluble in water or in ethanol (99.5)

Identification (1) Determine absorption spectra of solutions of Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS in methanol (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, recrystallize Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS, filter and dry the crystals, and perform the test with the crystals.

(3) A gruel-like liquid of Atorvastatin Calcium Hydrate prepared by adding a small amount of dilute hydrochloric acid responds to the Qualitative Tests (1) for calcium salt. A solution of Atorvastatin Calcium Hydrate in a mixture of methanol and water (7 : 3) (1 in 250) responds to the Qualitative Tests (3) for calcium salt.

Specific Optical Rotation $[\alpha]_D^{25}$: -7 ~ -10° (0.2 g, calculated on the anhydrous basis, dimethylsulfoxide, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Atorvastatin Calcium Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 20 mg of Atorvastatin Calcium Hydrate in a mixture of water and acetonitrile (1 : 1) to make 20 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of water and acetonitrile (1 : 1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to atorvastatin, obtained from the test solution is not larger than 0.3 times the peak area of atorvastatin from the standard solution, the area of the peak other than atorvastatin and peak mentioned above from the test solution is not larger than 0.1 times the peak area of atorvastatin from the standard solution, and the total area of the peaks other than atorvastatin from the test solution is not larger than the peak area of atorvastatin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust the pH to 5.0 with ammonia solution (28), and add water to make 1000 mL. To 400 mL of this solution add 100 mL of acetonitrile and 100 mL of tetrahydrofuran.

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (1 : 1).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-40	93	7
40-80	93→60	7→40

Flow rate: Adjust the flow rate so that the retention time of atorvastatin is about 16 minutes.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile (1 : 1) to make exactly 100 mL. Confirm that the peak area of atorvastatin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5 % of that with the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atorvastatin are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atorvastatin is not more than 2.0 %.

Time span of measurement: About 5 times as long as the retention time of atorvastatin, beginning after the solvent peak.

Isomer Dissolve 10 mg of Atorvastatin Calcium Hydrate in 2.0 mL of methanol, add 2.0 mL of ethanol (99.5) and hexane to make exactly 10 mL, and use this solution as the test solution. Perform the test with 20 μL of the test solutio as directed under Liquid Chromatography according to the following condition, and determine the amount of atorvastatin related substance I: not more than 3.0 %.

$$\text{Amount(\%) of atorvastatin related substance I} = \frac{A_i}{A_T} \times 100$$

A_i: Peak area of atorvastatin related substance I.

A_T: Sum of the peak areas of atorvastatin related substance I and atorvastatin.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with silica gel for liquid chromatography (5 to 10 μm in particle diameter) coated with amylose tris-3,5-dimethylphenylcarbamate.

Mobile phase: A mixture of hexane, ethanol (99.5), and trifluoroacetic acid (940 : 60 : 1).

Flow rate: 1.0 mL/minute.

System suitability

System performance: Dissolve a suitable amount each of Atorvastatin Calcium RS and Atorvastatin Related Substance I RS in methanol so that each mL contains 5 mg and 37.5 μg, respectively. To 2.0 mL of this solution add 2.0 mL of ethanol (99.5) and hexane to make exactly 10 mL, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution under the above operating conditions, the resolution between the peaks of atorvastatin related substance I and atorvastatin is not less than 2.0.

Water 3.5 ~ 5.5 % (50 mg, coulometric titration).

Assay Weigh accurately about 20 mg each of Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS (separately determine the water in the same manner as Atorvastatin Calcium Hydrate), dissolve each in a mixture of water and acetonitrile (1 : 1), add exactly 10 mL of the internal standard solution, then add a mixture of water and acetonitrile (1 : 1) to make 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$) = Amount (mg) of Atorvastatin Calcium RS, calculated on the anhydrous basis $\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of water and acetonotrile (1 : 1) (1 in 1500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust the pH to 4.0 with ammonia solution (28), and add water to make 1000 mL. To 530 mL of this solution add 270 mL of acetonitrile and 200 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of atorvastatin is about 10 minutes.

System suitability—

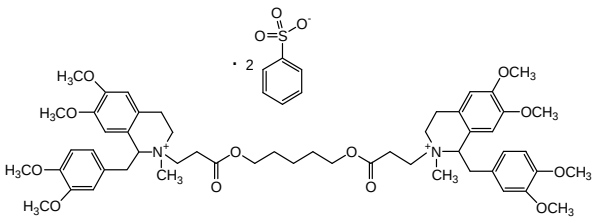
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Atracurium Besylate



$C_{53}H_{72}N_2O_{12}\cdot 2C_6H_5O_3S_2$: 1243.48

2,2'-{1,5-Pentanediylbis[oxy(3-oxo-3,1-propanediyl)]}bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroiso-quinolinium] dibenzenesulphonate [*64228-81-5*]

Atracurium Besylate contains not less than 96.0 % and not more than 102.0 % of atracurium besylate ($C_{53}H_{72}N_2O_{12}\cdot 2C_6H_5O_3S_2$), calculated on the anhydrous basis.

Description Atracurium Besylate is a white to yellowish-white solid.

Atracurium Besylate is very soluble ethanol (95), in acetonitrile, or in dichloromethane, and soluble in water.

Atracurium Besylate is hygroscopic.

Identification (1) Determine the infrared spectra of

Atracurium Besylate and Atracurium Besylate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The 3 principal isomeric peaks from test solution are same in retention time as those from standard solution in Assary.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Atracurium Besylate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Methyl benzenesulfonate***—Weigh accurately 0.10 g of Atracurium Besylate, dissolve in the mobile phase A int the Assay to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 20.0 mg of Methyl Benzenesulfonate RS, dissolve in acetonitrile to make exactly 100 mL, pipet 1.0 mL of this solution and add the mobile phase A to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the peak area of methyl benzenesulfonate from the test solution is not greater than that from the standard solution (0.01 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 217 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with base-deactivated octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A and mobile phase B: Prepare as directed in the Assay.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	80	20
0-5	80	20
5-15	80→75	20→25
15-25	75	25
25-30	75→55	25→45
30-38	55→0	45→100
38-45	0	100

Flow rate: 1.0 mL/minute

System suitability

System performance: Pipet 1 mL of the test solution and 5 mL of 0.02 % methyl benzenesulfonate in acetonitrile and add mobile phase to make 100 mL. When the procedure is run with this solution under the above operating condition, the resolution between the trans-trans isomer and methyl benzenesulfonate is not less than 12.0.

System repeatability: When the test is repeated 2 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 12 %.

(3) ***Toluene***—Weigh accurately about 0.20 g of Atracurium Besylate, dissolve in acetonitrile to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 0.1 g of Atracurium Besylate RS, dissolve in acetonitrile to make exactly 100 mL, pipet 5.0 mL of this solution, add acetonitrile to make exactly 50 mL and use this solution as the standard solution. Perform the test with 1 μL of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions: the area of toluene peak from the test solution is not greater than that from the standard solution (not more than 0.5 %).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica analytical column 0.53 mm in internal diameter and 30 m in length, coated the inner surface with 5 % phenyl-95 % methyl polysiloxane for Gas Chromatography 5 μm in thickness. A fused-silica guard column 0.53 mm in internal diameter and 5 m in length, coated the inner surface with deactivated with phenylmethyl siloxane.

Column temperature: Maintain 35 ºC for 5 minutes after injection of the sample, then increase to 175 ºC at a rate of 8 ºC per minute, then increase to 260 ºC at a rate of 35 ºC per minute, and maintain 260 ºC for at least 16 minutes.

Injection port temperature: A constant temperature of about 70 ºC.

Detector temperature: A constant temperature of about 260 ºC.

Carrier gas: Helium

Flow rate: 35 mL/second

System suitability

System performance: Weigh accurately each of dichloromethane, 1,4-dioxane, trichloroethylene, and chloroform and dissolve in acetonitrile to make the solution that contains 12.0, 7.6, 1.6, and 1.2 μg per mL, respectively. When the procedure is run with 1 μL of this solution under the above operating conditions, the resolution between two peaks is not less than 1.0.

System repeatability: Weigh accurately each of dichloromethane, 1,4-dioxane, trichloroethylene, and chloroform and dissolve in acetonitrile to make the solution that contains 12.0, 7.6, 1.6, and 1.2 μg per mL, respectively. When the test is repeated with 1 μL each of this solution under the above operating conditions, the relative standard deviation of each peak area is not more than 15 %.

(4) ***Related substances***—Use the test solution of the Assay as the test solution. To 1.0 mL of the stand-

ard solution of the Assay add mobile phase A of the Assay to make 100 mL and use this solution as the standard solution. Perform the test with 20 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and measure all the peak areas, except the three main isomeric peaks. Calculate the percentage of each related substances in the portion of Atracurium Besylate taken by the formula: the amount of laudanosine having the relative retention time of about 0.3 is not more than 0.5 %, the amount of each of the other related substances is not more than 1.0 %, and the total amount of related substances is not more than 3.5 %. Use the peak area of laudanosine after dividing by its relative response factor, 1.9.

$$\text{Amount (\%) of each related substance} = 10000 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

A_i: Peak area for each related substances obtained from the test solution

A_S: Peak area for the *cis-cis* isomer obtained from the standard solution

C: Concentration (mg/mL) of the *cis-cis* isomer in the standard solution.

W: Amount (mg) of Atracurium Besylate taken.

System suitability

When the test is repeated at least 2 times with 20 μL each of the standard solution under the operating conditions of the Assay, the relative standard deviation of the peak area of *cis-cis* isomer is not more than 10 %.

Water Not more than 5.0 % (1 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g each of Atracurium Besylate and Atracurium Besylate RS, dissolve in mobile phase A to make exactly 100 mL and use the solutions as the test solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine total area, A_T and A_S, of the three isomeric peaks, respectively.

$$\text{Amount (mg) of atracurium besylate } (C_{53}H_{72}N_2O_{12}\cdot 2C_6H_5O_3S_2) = \text{Amount (mg) of Atracurium Besylate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with base-deactivated octadecylsilanized silica gel for Liquid Chromatography (5 to 10 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: The mixture of buffer solution, acetonitrile and methanol (75 : 20 : 5)

Mobile phase B: The mixture of buffer solution, methanol and acetonitrile (50 : 30 : 20)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	80	20
0-5	80	20
5-15	80→40	20→60
15-25	40	60
25-35	40→0	60→100

Flow rate: 1.0 mL/minute

System suitability

System performance: When the procedure is run with the standard solution under the above operating condition, the relative retention times are about 0.8, 0.9, and 1.0 for the *trans-trans* isomer, the *cis-trans* isomer, and the *cis-cis* isomer, respectively. The resolution between the *trans-trans* isomer and the *cis-trans* isomer and between the *cis-trans* isomer and the *cis-cis* isomer is not less than 1.1.

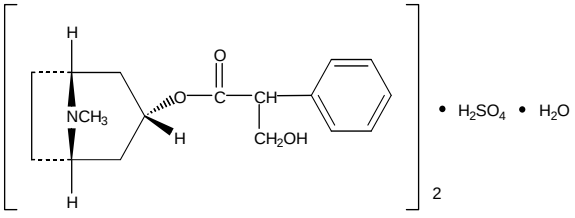
System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of each isomer is not more than 2.0 %.

Buffer solution—Weigh about 10.2 g of monobasic potassium phosphate, and dissolve in about 950 mL of water, while stirring, adjust with phosphoric acid to a pH of 3.1, add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Atropine Sulfate Hydrate



$[(C_{17}H_{23}NO_3)_2.H_2SO_4\cdot H_2O$: 694. 83]

bis[(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) 3-hydroxy-2-phenylpropanoate] sulfate monohydrate [*5908-99-6*]

Atropine Sulfate Hydrate, when dried, contains not less than 98.0 % and not more than 101.0 % of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$: 676.82]

Description Atropine Sulfate Hydrate appears as colorless crystals or a white, crystalline powder and is odorless.
Atropine Sulfate Hydrate is very soluble in water or in acetic acid (100), freely soluble in ethanol (95) and practically insoluble in ether.
Melting point—188 ~ 194 ºC (with decomposition). Introduce a capillary tube charged with dried sample into a bath previously heated to 180 ºC and continue to heat at the rate of rise of about 3 ºC per minute.
Atropine Sulfate Hydrate is affected by light.

Identification (1) To 1 mg of Atropine Sulfate Hydrate, add 3 drops of fuming nitric acid and evaporate the mixture in a water-bath to dryness. Dissolve the residue in 1 mL *N,N*-dimethylformamide and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) To 2 mL of a solution of Atropine Sulfate Hydrate (1 in 50), add 4 to 5 drops of hexachloroplatinic (IV) acid TS: a lusterless, yellowish white precipitate is formed.

(3) To 5 mL of a solution of Atropine Sulfate Hydrate (1 in 25), add 2 mL of ammonia TS and allow to stand for 2 to 3 minutes. Collect the precipitate, wash with water and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts between 115 ºC and 118 ºC.

(4) A solution of Atropine Sulfate Hydrate (1 in 20) responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{25}$: -0.60 ~ +0.05° (1 g, water, 20 mL)

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Atropine Sulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Acid***—Dissolve 1.0 g of Atropine Sulfate Hydrate in 20 mL of water and add 0.30 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) ***Related Substances***—Dissolve 0.25 g of Atropine Sulfate Hydrate in 1 mL of diluted hydrochloric acid (1 in 10), add water to make 15 mL and use this solution as the test solution. (i) To 5 mL of the test solution, add 2 to 3 drops of hexachloroplatinic (IV) acid TS: no precipitate is formed. (ii) To 5 mL of the test solution, add 2 mL of ammonia TS and shake vigorously: the turbidity of the solution is not greater than that of the following control solution.

Control solution—To 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 50 mL. To this solution, add 1 mL of silver nitrate TS and allow 7 mL of the mixture to stand for 5 minutes.

(4) ***Hyoscyamine***—Weigh accurately about 1 g of Atropine Sulfate Hydrate, previously dried and dissolve in water to make exactly 10 mL: the specific optical rotation $[\alpha]_D^{20}$ of this solution in a 100 mm cell is between -0.60° and +0.10°.

(5) ***Readily carbonizable substances***—Take 0.20 g of Atropine Sulfate Hydrate and perform the test: the solution has no more color than Color Matching Fluid A.

Loss on Drying Not more than 4.0 % (0.5 g, in vacuum, P_2O_5, 110 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 0.25 g of Atropine Sulfate Hydrate, previously dried and accurately weighed, in 30 mL of acetic acid (100). If necessary, dissolve by warming and cool. Titrate with 0.05 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 33.841 mg of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Atropine Sulfate Injection

Atropine Sulfate Injection is an aqueous solution for injection. Atropine Sulfate Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$: 694.83].

Method of Preparation Prepare as directed under Injections, with Atropine Sulfate Hydrate.

Description Atropine Sulfate Injection is a clear, colorless liquid.

pH — 4.0 ~ 6.0.

Identification (1) Evaporate a volume of Atropine Sulfate Injection, equivalent to 1 mg of Atropine Sulfate Hydrate according to the labeled amount, in a water-bath to dryness. Proceed with the residue as directed in the Identification (1) under Atropine Sulfate Hydrate.

(2) Evaporate an exactly measured volume of Atropine Sulfate Injection, equivalent to 5 mg of Atropine Sulfate Hydrate according to the labeled amount, on a

water bath to dryness. After cooling, dissolve the residue in 1 mL of ethanol (95), and use this solution as the test solution. If insoluble substance remains, crush it, allow to stand, and use the clear supernatant liquid as the test solution. Separately, dissolve 10 mg of Atropine Sulfate Hydrate RS in 2 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90 : 7 : 3) to a distance of about 10 cm, and dry the plate at 80 ºC for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: the spots obtained from the test solution and the standard solution show an orange color and the same R_f value.

(3) Atropine Sulfate Injection responds to the Qualitative Tests for sulfate.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 75 EU/mg of Atropine Sulfate Hydrate

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay To an exactly measured volume of Atropine Sulfate Injection, equivalent to about 5 mg of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4{\cdot}H_2O]$, add exactly 3 mL of the internal standard solution and water to make 50 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Atropine Sulfate Hydrate RS (determine previously its loss on drying in the same manner as directed under Atropine Sulfate Hydrate), and add water to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of atropine to that of the internal standard for the test solution and the standard solution.

$$\text{Amount (mg) of atropine sulfate hydrate } [(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4{\cdot}H_2O] = W_S \times \frac{Q_T}{Q_S} \times \frac{1}{5} \times 1.0266$$

W_S: Amount(mg) of Atropine Sulfate RS, calculated based on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 1000).

Operating conditions

Detector: A ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: To 0.4 g of sodium lauryl sulfate add 500 mL of diluted phosphoric acid (1 in 1000) to dissolve, and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 16 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolutions between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of atropine to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Atropine Sulfate Tablets

Atropine Sulfate Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4\ H_2O$: 694.83].

Method of Preparation Prepare as directed under Tablets, with Atropine Sulfate hydrate.

Identification (1) Weigh a portion of finely powdered Atropine Sulfate Tablets, equivalent to about 1 mg of Atropine Sulfate Hydrate according to the labeled amount, add 1 drop of ammonia solution (28) and 2 mL of chloroform, mix, separate the chloroform layer and evaporate the chloroform on a water-bath. Proceed with the residue as directed in the Identification (1) under Atropine Sulfate hydrate.

(2) Weigh a portion of finely powdered Atropine Sulfate Tablets, equivalent to about 5 mg of Atropine Sulfate Hydrate according to the labeled amount, shake with 5 mL of ethanol (95), filter through glass filter (G4) and use this filtrate as the test solution. Separately,

weigh accurately about 50 mg of Atropine Sulfate Hydrate RS, add ethanol (95) to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 50 μL of the test solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the chromatogram with a mixture of chloroform and diethyl amine (9 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly hexachloroplatinic (IV) acid-potassium iodide TS on the plate: the spots obtained from the test solution and the standard solution show a purple color and the same R_f value.

(3) Atropine Sulfate Tablets respond to the Qualitative Tests for sulfate.

Disintegration Test It meets the requirement, provided that the time limit of the test is 15 minutes.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Atropine Sulfate Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$], transfer to a separator in 5 mL of a pH 9.0 buffer solution and add 2.0 mL of the internal standard solution. Adjust with 1 mol/L sodium hydroxide to a pH of 9.0 and extract with two 10 mL volumes of dichloromethane. Filter the dichloromethane layer through 1 g of anhydrous sodium sulfate on a pledget of absorbent cotton, evaporate the filtrate with the aid of nitrogen current to dryness, dissolve the residue in 2.0 mL of dichloromethane and use this solution as the test solution. Separately, weigh accurately about 10 mg of Atropine Sulfate Hydrate RS, previously dried at 120 ºC for 4 hours and dissolve in water to make exactly 100 mL. Prepare this solution before use. Transfer exact 10 mL of this solution to a separator, add 2.0 mL of the internal standard solution and 5.0 mL of a pH 9.0 buffer solution and adjust to a pH of 9.0 with 1 mol/L sodium hydroxide. Extract with two 10 mL volumes of dichloromethane, filter the extracts through 1 g of anhydrous sodium sulfate on a pledget of absorbent cotton, evaporate the filtrate with the aid of nitrogen current to dryness, dissolve the residue in 2.0 mL of dichloromethane and use this solution as the standard solution. Perform the test with 1 μL each of the test and the standard solution as directed under Gas Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of atropine sulfate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of atropine sulfate hydrate } [(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O] = \frac{W}{10} \times \frac{Q_T}{Q_S} \times 1.0266$$

W: Amount (mg) of Atropine Sulfate Hydrate RS, calculated on the anhydrous basis.

Internal standard solution—A solution of homatropine hydrobromide (5 in 10000). Prepare this solution before use.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 2 mm in internal diameter and about 1.8 m in length, having 50 % phenyl- and 50 % methyl-polysiloxane coated at the ratio of 3 % on siliceous earth.

Column temperature: About 225 ºC.

Carrier gas: Nitrogen.

Flow rate: 25 mL/minute.

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions with the resolution. being not less than 4.0 and with the symmetry factor being not more than 2.0.

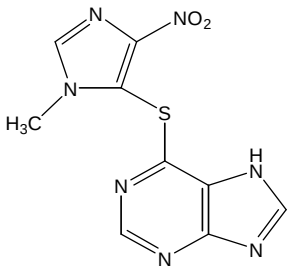
System repeatability: When the test is repeated 6 times with 1 μL each of the standard solution under the above operating conditions,the relative standard deviation of the ratio of the peak area is not more than 2.0 %.

pH 9.0 Phosphate buffer solution—Dissolve 34.8 g of dipotassium hydrogen phosphate in 900 mL of water and adjust to pH 9.0 with 3 mol/L hydrochloric acid or 1 mol/L sodium hydroxide solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Azathioprine



$C_9H_7N_7O_2S$: 277.26

6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)sulfa-nyl]-7*H*-purine [*446-86-6*]

Azathioprine, when dried, contains not less than 98.5 % and not more than 101.0 % of azathioprine ($C_9H_7N_7O_2S$).

Description Azathioprine appears as pale yellow

crystals or crystalline powder and is odorless.
Azathioprine is sparingly soluble in *N,N*-dimethylformamide or in pyridine, very slightly soluble in water or in ethanol (99.5), and practically insoluble in chloroform or in ether.
Azathioprine dissolves in sodium hydroxide TS or in ammonia TS.
Azathioprine is gradually colored by light.

Melting point—About 240 ºC (with decomposition).

Identification (1) Dissolve 10 mg of Azathioprine in 50 mL of water by warming. To 5 mL of this solution, add 1 mL of dilute hydrochloric acid and 10 mg of zinc powder and allow to stand for 5 minutes: a yellow color is produced. Filter this solution: the filtrate responds to the Qualitative Tests for primary aromatic amines and a red color is produced.

(2) Dissolve 10 mg of Azathioprine in 50 mL of water by warming. To 1 mL of this solution, add 0.5 mL of phosphotungstic acid TS and 0.5 mL of dilute hydrochloric acid: a white precipitate is formed.

(3) Prepare the test solution by proceeding with 30 mg of Azathioprine according to the Oxygen Flask Combustion Method, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests (1) for sulfate.

(4) Dissolve 10 mg of Azathioprine in 100 mL of 2 mol/L hydrochloric acid TS. To 5 mL of this solution, add water to make 50 mL and determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotomtry: it exhibits a maximum between 278 nm and 282 nm.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Azathioprine in 50 mL of *N,N*-dimethylformamide: the solution is clear and shows a pale yellow color.

(2) ***Acid or alkali***—Add 100 mL of water to 2.0 g of Azathioprine, shake well for 15 minutes, centrifuge for 5 minutes at 10000 revolutions per minute and filter. Discard the first 20 mL of the filtrate, add 2 drops of methyl red TS to 40 mL of the subsequent filtrate and use this solution as the test solution. (i) Add 0.10 mL of 0.02 mol/L hydrochloric acid VS to 20 mL of the test solution: a red color develops. (ii) Add 0.10 mL of 0.02 mol/L sodium hydroxide VS to 20 mL of the test solution: a yellow color develops.

(3) ***Sulfate***—To 25 mL of the filtrate obtained in (2), add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Azathioprine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Azathioprine, according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 10 mg of Azathioprine in 80 mL of the mobile phase by warming, cool, add the mobile phase to make 100 mL and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than that of Azathioprine from the test solution is not larger than 1/2 of the peak area of Azathioprine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 296 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Adjust to a pH of 2.5 by adding diluted phosphoric acid (3 in 2000) to diluted 0.05 mol/L monobasic potassium phosphate TS (1 in 2). To 800 mL of this solution, add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of Azathioprine is about 8 minutes.

System suitability

Detection sensitivity: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Adjust the detection sensitivity so that the peak area of azathioprine obtained from 20 μL of this solution is between 8 and 12 % of that of azathioprine obtained from 20μL of the standard solution.

System performance: Dissolve 10 mg of Azathioprine in 80 mL of water by warming, cool and add water to make 100 mL. To 2 mL of this solution add 2 mL of a solution, separately prepared by dissolving 60 mg of benzoic acid in 3 mL of methanol and diluting with water to make 10 mL, and add the mobile phase to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, azathioprine and benzoic acid are eluted in this order with the resolution between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL each of this solution under the above operating conditions, the relative standard of the peak areas of azathioprine is not more than 2.0 %.

Time span of measurement: About three times as long as the retention time of Azathioprine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Azathioprine,

previously dried, add 80 mL of *N,N*-dimethylformamide and warm to dissolve. After cooling, titrate with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue-green (indicator: 1 mL of thymol blue-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 27.726 mg of $C_9H_7N_7O_2S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Azathioprine Tablets

Azathioprine Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of azathioprine ($C_9H_7N_7O_2S$: 277.26).

Method of Preparation Prepare as directed under Tablets, with Azathioprine.

Identification (1) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 10 mg of Azathioprine according to the labeled amount. Add 50 mL of water, shake well while warming and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Azathioprine.

(2) Proceed with 1 mL of the filtrate obtained in (1) as directed in the Identification (2) under Azathioprine.

(3) Determine the absorption spectrum of the test solution in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 278 nm and 282 nm.

(4) Weigh a portion of powdered Azathioprine Tablets, equivalent to 0.1 g of Azathioprine to the labeled amount. Add 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), shake well, filter and use the filtrate as the test solution. Separately, dissolve 0.1 g of Azathioprine RS in 10 mL of a solution of ammonia solution (28) in methanol (1 in 10) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, a solution of ammonia solution (28) in methanol (1 in 10), *n*-butyl formate and 1,2-dichloroethane (15 : 10 : 5 : 2) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the test solution and the standard solution show the same R_f value.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

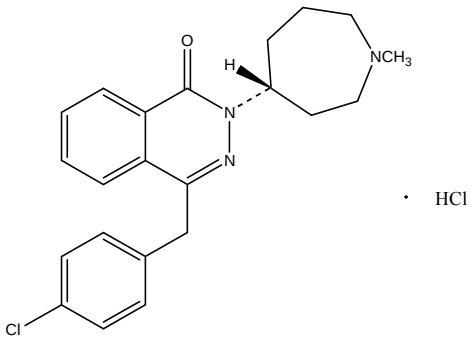
Assay Weigh accurately and powder not less than 20 Azathioprine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of azathioprine ($C_9H_7N_7O_2S$), add 20 mL of dimethylsulfoxide for Spectrophotometry, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Azathioprine RS, previously dried at 105 ºC for 5 hours, dissolve in 20 mL of dimethylsulfoxide for spectrophotometry and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Measure exactly 3 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 280 nm, respectively as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of azathioprine } (C_9H_7N_7O_2S) = \text{Amount (mg) of Azathioprine RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Azelastine Hydrochloride



· HCl

and enantioner

$C_{22}H_{24}ClN_3O$·HCl : 418.36

4-[(4-Chlorophenyl)methyl]-2-(1-methylazepan-4-yl)phthalazin-1-one hydrochloride [*79307-93-0*]

Azelastine Hydrochloride contains not less than 98.5 % and not more than 101.0 % of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl), calculated on the dried basis.

Description Azelastine Hydrochloride is a white crystalline powder.
Azelastine Hydrochloride is soluble in ethanol (99.5), slightly soluble in water, and practically insoluble in ether, in *n*-hexane, or in toluene.

Identification (1) Determine the infrared spectra of Azelastine Hydrochloride and Azelastine Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Azelastine Hydrochloride in water (1 in 50) responds to the Qualitative Tests (2) for chlorides.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.05 ~ +0.50º (previously dried, 1.0 g, dichloromethane, 20 mL, 100 mm)

Purity (1) ***Acidity or alkalinity***—To 10 ml of solution of Azelastine Hydrochloride in water (1 in 100) add 0.2 ml of bromthymol blue solution. Not more than 0.1 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS is required to change the color of the solution.

(2) ***Heavy metals***—Proceed with 1.0 g of Azelastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Azelastine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) ***1-Methyl-4-(2-benzoylhydrazino)azepane***—Weigh accurately 0.1 g of Azelastine Hydrochloride and dissolve in 12 mL of methanol and add water to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately 5.0 mg of 1-Methyl-4-(2-benzoylhydrazino)azepane Hydrochloride RS and dissolve in 60 % methanol to exactly 100 mL. Pipet 5.0 mL of this solution and dilute in 60 % methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of 1-methyl-4-(2-benzoylhydrazino) azepane from the test solution is not larger than that of principal peak from the standard solution. (not more than 0.1 %)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (10 μm in particle diameter).

Mobile phase: A 0.02 mol/L solution of sodium octanesulfonate in the mixture of methanol, water and acetic acid (100) (60 : 40 : 1).

Flow rate: 1.5 mL/minute.

(5) ***Related substances***—Weigh accurately 50 mg of Azelastine Hydrochloride, dissolve in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than azelastine from the test solution is not larger than 1/10 times the peak area of azelastine from the standard solution, and the total area of the peaks other than azelastine from the test solution is not larger than 1/2 times the peak area of azelastine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of water, acetonitrile, and perchloric acid (660 : 340 : 1)

Flow rate: Adjust the flow rate so that the retention time of azelastine is about 10 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the system suitability solution. Confirm that the peak area of azelastine obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of azelastine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of azelastine is not more than 1.0 %.

Loss on Drying Not more than 1.0 % (1g, 105 °C, constant mass).

Residue on Ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 0.3 g of Azelastine Hydrochloride, dissolve in 5 ml of anhydrous formic acid, add 30 ml of acetic anhydride and titrate quickly with 0.1 mol/L perchloric acid VS (potentiometric ti-

tration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.84 mg of $C_{22}H_{24}ClN_3O \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Azithromycin Capsules

Azithromycin Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.99).

Method of Preparation Prepare as directed under Capsules, with Azithromycin.

Identification (1) The retention time of the principal peak obtained from the test solution corresponds to that of the standard solution, as obtained in the Assay.

(2) Weigh accurately a suitable amount each of the contents of Azithromycin Capsules and Azithromycin RS, dissolve each in a mixture of chloroform and methanol (1 : 1) so that each mL contains 1 mg (potency), and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 50 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, and diethylamine (150 : 50 : 20), and heat at 100 °C for 10 minutes. Spray evenly a solution prepared by dissolving 3 g of vanillin in 100 mL of ethanol (95) on the plate, and heat again at 100 °C for 10 minutes: the black spots obtained from the test solution and standard solution have the same R_f value.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Azithromycin Capsules at 100 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of pH 6.0 phosphate buffer solution as the dissolution solution. Take not less than 20 mL of the dissolved solution 45 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately about 15 mg (potency) of Azithromycin RS, and dissolve in the dissolution solution to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 25 mL, pipet 4 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed in the Assay under Azithromycin Hydrate. The dissolution rate of Azithromycin Capsules in 45 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.98)
= Amount [mg (potency)] of Azithromycin RS

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 23.04$$

C: Labeled amount [mg (potency)] of azithromycin ($C_{38}H_{72}N_2O_{12}$) in 1 capsule

pH 6.0 Phosphate buffer solution—Adjust the pH of 6000 mL of 0.1 mol/L sodium hydrogen phosphate to 4.4 ± 0.1 with about 40 mL of hydrochloric acid, add 600 mg of trypsin, and mix.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 20 Azithromycin Capsules, and powder if necessary. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) according to the labeled potency, and dissolve in acetonitrile so that each mL contains 1 mg (potency). Pipet 2 mL of this solution, add the internal standard solution to make exactly 50 mL, pipet 2 ml of this solution, add the internal standard solution ot make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 33 mg (potency) of Azithromycin RS, dissolve in acetonitrile to make exactly 200 mL, pipet 2 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azithromycin to that of the internal standard.

Amount [μg (potency)] of azithromycin ($C_{38}H_{72}N_2O_{12}$)
= Amount [μg (potency)] of Azithromycin RS

$$\times \frac{Q_T}{Q_S} \times \frac{25}{8}$$

Internal standard solution—Adjust the pH of a mixture of 0.02 mol/L potassium dihydrogen phosphate solution and acetonitrile (71 : 29) to 8.0 with 10 mol/L potassium hydroxide TS. Weigh accurately about 1.5 mg of diphenhydramine hydrochloride, and dissolve in this solution to make 1000 mL.

Operating conditions

Detector: An electrochemical detector

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with alumina coated with polybutadiene.

Mobile phase: Adjust the pH of a mixture of 0.02 mol/L potassium dihydrogen phosphate solution and acetonitrile (71 : 29) to 11.0 with 10 mol/L potassium hydroxide TS.

Flow rate: 1.5 mL/minute

Containers and Storage ***Containers***—Tight containers.

Azithromycin for Syrup

Azithromycin for Syrup is a preparation for syrup, which is suspended before use.
Azithromycin for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.99).

Method of Preparation Prepare as directed under Syrups, with Azithromycin Hydrate.

Identification (1) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(2) Proceed as directed in the Identification (2) under Azithromycin Capsules.

pH The pH of a solution obtained by dissolving an amount of Azithromycin for Syrup, equivalent to 0.4 g (potency) of azithromycin, in 10 mL of water is between 9.0 and 11.0.

Water Not more than 1.5 % (0.5 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately a suitable amount of Azithromycin for Syrup, dissolve in a mixture of 0.04 mol/L dipotassium hydrogen phosphate TS (pH 8.0) and acetonitrile (4 : 6) so that each mL contains 0.5 mg (potency), and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Azithromycin RS, dissolve in a mixture of 0.04 mol/L dipotassium hydrogen phosphate (pH 8.0) and acetonitrile (4 : 6) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following ocnditoins, and determine the peak areas, A_T and A_S, of azithromycin in the test solution and standard solution.

Amount [μg (potency)] of azithromycin ($C_{38}H_{72}N_2O_{12}$)

$$= \text{Amount [μg (potency)] of Azithromycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized polyvinyl alcohol for liquid chromatography (5 μm in particle diameter).

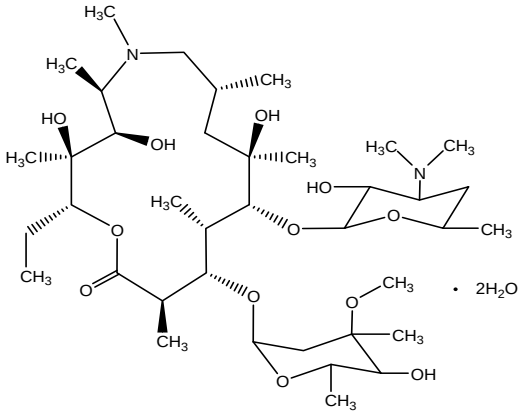
Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 6.7 g of dipotassium hydrogen phosphate in water to make exactly 1000 mL, and adjust the pH to 11.0 with 10 mol/L potassium hydroxide TS. To 400 mL of this solution add 600 mL of acetonitrile, and mix.

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Azithromycin Hydrate



Azithromycin $C_{38}H_{72}N_2O_{12}\cdot 2H_2O$: 785.02

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethyl-amino)-β-D-xylo]oxy}-1-oxa-6-azacyclo-pentadec-13-yl 2,6-dideoxy-3-C-methyl-3- O-methyl-α-L-ribo-hexopyranoside dihydrate [*117772-70-0*]

Azithromycin Hydrate is a derivative of erythromycin.
Azithromycin Hydrate contains not less than 945 μg (potency) and not more than 1030 μg (potency) per mg of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.98), calculated on the anhydrous basis.

Description Azithromycin Hydrate appears as white crystalline powder.
Azithromycin Hydrate is freely soluble in methanol or in ethanol (99.5), and practically insoluble in water.

Identification Determine the infrared spectra of Azithromycin Hydrate and Azithromycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement, except, when it is amorphous and when most of the particles show no birefringence or extinction positions.

Specific Optical Rotation $[\alpha]_D^{20}$: -45 ~ -49°(0.4 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.2 g of Azithromycin Hydrate in 10 mL of 50 % methanol solution is between 9.0 and 11.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Azithromycin Hydrate to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Total related substances***—Perform the test according to the procedure of Assay. Prepare the test solution and as follows. Weigh accurately about 33 mg of Azithromycin Hydrate, dissolve in 5 mL of acetonitrile, add the mixture of 0.02 mol/L potassium dihydrogen phosphate solution and acetonitrile (71:29), pH 8.0 adjusted with 10 mol/L potassium hydroxide to make 100 mL, and use this solution as the test solution. Perform the test with 50 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions, and obtain the peak areas of azithromycin and each related substance (not more than 5.5 %).

$$\text{Amount [\%] of total related substances} = \frac{\text{total peak areas other than the principal peak}}{\text{total peak areas}} \times 100$$

Operating conditions

Mobile phase: Adjust the pH of the mixture of 0.02 mol/L of potassium dihydrogen phosphate solution and acetonitrile (75:25) to 11.0 with 10 mol/L potassium hydroxide TS.

Flow rate: 1.2 mL per minute.

Time span of measurement: About 80 minutes.

(3) ***Erythromycin A iminoether***—Weigh accurately an appropriate amount of Azithromycin Hydrate, dissolve in the mixture of methanol and chloroform (1:1) to make the solution so that each mL contains 25 mg, and use this solution as the test solution. Separately, weigh accurately 2.5 mg (potency) of Erythromycin A iminoether RS, dissolve in the mixture of methanol and chloroform (1:1), to make the solution so that each mL contains 0.125 mg, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 50 μL of each solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with the mixture of ethylacetate, hexane and diethylamine (15:5:2) as the developing solvent mixture, and heat the plate at 100 °C for 10 minutes. Spray evenly the color developing solution on the plate and heat the plate at 100 °C. The color developing solution mixture is prepared by dissolving 3 g of vaniline in 100 mL of ethanol (95) and adding 1.5 mL of sulfuric acid. The spots other than the principal spot obtained from the test solution are not more intense than the principal spot from the standard solution.

Water Not less than 4.0 % and not more than 5.0 % (0.4 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg (potency) each of Azithromycin Hydrate and Azithromycin RS, dissolve each in a mixture of acetonitrile and water (3 : 2), add exactly 2 mL of the internal standard solution and a mixture of acetonitrile and water (3 : 2) to make 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of azithromycin to that of the internal standard.

$$\text{Amount [μg (potency)] of azithromycin } (C_{38}H_{72}N_2O_{12}) = \textit{Amou}\text{nt [μg (potency)] of Azithromycin Hydrate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 4,4'-bis(diethylamino)benzophenone in acetonitrile (3 in 4000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 6.97 g of dipotassium hydrogen phosphate in 750 mL of water, adjust the pH to 11.0 with potassium hydroxide TS, and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of azithromycin is about 10 minutes.

System suitability

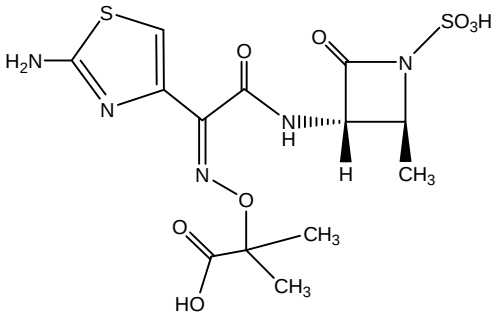
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, azithromycin and the internal stand-

ard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of azithromycin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Aztreonam



$C_{13}H_{17}N_5O_8S_2$: 435.43

2-({[(1*Z*)-1-(2-Amino-1,3-thiazol-4-yl) -2-{[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-methyl-propanoic acid [*78110-38-0*]

Aztreonam contains not less than 920 μg (potency) and not more than 1030 μg (potency) per mg of aztreonam ($C_{13}H_{17}N_5O_8S_2$: 435.43), calculated on the anhydrous basis.

Description Aztreonam appears as white to yellowish white crystalline powder.
Aztreonam is freely soluble in dimethylsulfoxide, slightly soluble in water or in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Aztreonam and Aztreonam RS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Aztreonam in deuterated dimethylsulfoxide (1 in 10) as directed under Nuclear Magnetic Resonance Spectrophotometry: it exhibits a multiple signal at around 1.5 ppm and a single signal at around 7.0 ppm. The ratio of the integrated intensity of each signal is 9 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: -26 ~ -32° (0.25 g calculated on the anhydrous basis, water, 50 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.1 g (potency) of Aztreonam in 20 mL of water is between 2.2 and 2.8.

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Aztreonam in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Aztreonam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Aztreonam according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately about 40 mg (potency) of Aztreonam, dissolve in water to make 100 mL, and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution: the area of each peak other than aztreonam from the test solution is not larger than the peak area of aztreonam from the standard solution, and the total area of the peaks other than aztreonam from the test solution is not larger than 2.5 times the peak area of aztreonam from the standard solution.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, add water to make exactly 10 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add water to make exactly 10 mL. Confirm that the peak area of aztreonam obtained from 25 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of aztreonam obtained from 25 μL of the standard solution is between 10 and 20 mm.

System performance: When the procedure is run with 25 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aztreonam is not more than 2.0 %.

Time span of measurement: About 4 times as long as the retention time of aztreonam beginning after the solvent peak.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (1 g)

Sterility Test It meets the requirement, when Aztreonam is used in a sterile preparation. Dissolve 8 g of arginine in 200 mL of the rinsing fluid, autoclave at 121 °C, and cool. To this solution add 10.0 g of Aztreonam, dissolve completely, and use this solution as the test solution.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of aztreonam, when Aztreonam is used in a sterile preparation.

Assay Weigh accurately about 20 mg (potency) each of Aztreonam and Aztreonam RS, dissolve each in 70 mL of water, add exactly 10 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aztreonam to that of the internal standard.

$$\text{Amount [μg (potency)] of aztreonam } (C_{13}H_{17}N_5O_8S_2) = \text{Amount [μg (potency)] of Aztreonam RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 1.7 g of tetrabutylammonium hydrogen sulfate in 300 mL of water, adjust the pH to 3.0 with 0.5 mol/L disodium hydrogen phosphate TS, and add water to make 1000 mL. To 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of aztreonam is about 8 minutes.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aztreonam to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Aztreonam for Injection

Aztreonam for Injection is a preparation for injection, which is dissolved before use.

Aztreonam for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$: 435.43)

Method of Preparation Prepare as directed under Injections, with Aztreonam.

Description Aztreonam for Injection appears as white to yellowish white masses or powder.

Identification (1) Dissolve an amount of Aztreonam for Injection, equivalent to 6 mg (potency) of aztreonam according to the labeled amount, in 1 mL of hydroxylammonium chloride-ethanol TS, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color is produced.

(2) Dissolve an amount of Aztreonam for Injection, equivalent to 3 mg (potency) of aztreonam according to the labeled amount, in 100 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 289 nm and 293 nm.

pH The pH of a solution obtained by dissolving an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of aztreonam, in 10 mL of water is between 4.5 and 7.0.

Purity ***Clarity of solution***—Dissolve an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of aztreonam according to the labeled amount, in 10 mL of water: the solution is clear, and its absorbance at 450 nm determined as directed under Ultraviolet-visible Spectrophotometry is not more than 0.06.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of aztreonam.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Take a number of containers of Aztreonam for Injection, equivalent to about 5 g (potency) of aztreonam, dissolve the contents of each in water, and transfer to a 100 mL volumetric flask. Wash each container with water, combine the washings and the solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Aztreonam RS, dissolve in water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Aztreonam.

Amount [mg (potency)] of aztreonam ($C_{13}H_{17}N_5O_8S_2$)
= Amount [mg (potency)] of Aztreonam RS

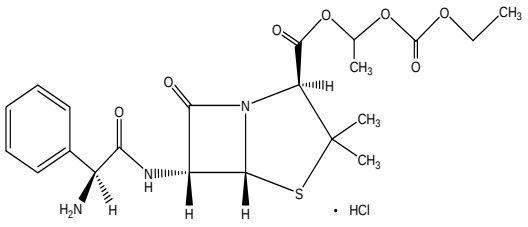
$$\times \frac{Q_T}{Q_S} \times 250$$

Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250)

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Bacampicillin Hydrochloride



$C_{21}H_{27}N_3O_7S \cdot HCl$: 501.98

1-Ethoxycarbonyloxyethyl(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrochloride [*37661-08-8*]

Bacampicillin Hydrochloride contains not less than ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41) 626 μg (potency) per mg of Bacampicillin Hydrochloride, calculated on the anhydrous basis.

Description Bacampicillin Hydrochloride is white to pale yellow crystalline powder, and has a characteristic odor.

Bacampicillin Hydrochloride is freely soluble in methanol or in ethanol (99.5) and soluble in water.

Identification (1) Determine the absorption spectra of solutions of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride RS in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bacampicillin (1 in 50) responds to the Qualitative Tests for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: +140 ~ +170° (0.1 g calculated on the anhydrous basis, ethanol (99.5), 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.2 g of Bacampicillin Hydrochloride in 10 mL of water is between 3.0 and 5.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Free ampicillin***— Weigh accurately about 0.1 g of Bacampicillin Hydrochloride, transfer into a 100 mL separator, add exactly 15 mL of ice-cold 0.05 mol/L phosphate buffer solution, pH 7.0, then add 25 mL of ice-cold chlorform, shake, and abandon the chloroform layer. Repeat the procedure twice with two 25 mL portions of ice-cold chloroform. Centrifuge the water layer, filter the clear supernatant liquid, and use the filtrate as the test solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to 20 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0 and water to make exactly 25 mL, and use this solution as the standard solution. To exactly 10 mL each of the test solution and the standard solution add exactly 2 mL of sodium hydroxide TS, allow to stand for exactly 15 minutes, add exactly 2 mL of 1 mol/L hydrochloric acid TS, exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6, and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 20 minutes without exposure to light. Titrate each of these solutions with 0.01 mol/L sodium thiosulfate VS until the color of the solution turns to colorless. Separately, to exactly 10 mL each of the test solution and the

standard solution add exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, and perform a blank determination with the same manner. Determine the consumed amounts (mL) of 0.005 mol/L iodine VS, V_T and V_S, of the test solution and the standard solution: the amount of ampicillin is not more than 1.0 %.

$$\text{Amount (\%) of free ampicillin } (C_{16}H_{19}N_3O_4S) = \frac{V_T}{V_S} \times \frac{\text{Amount (mg) of Ampicillin RS taken}}{\left[\text{Amount (mg) of Bacampicillin Hydrochloride taken}\right]} \times \frac{100}{20}$$

0.3 mol/L potassium hydrogen phthalate buffer solution (pH 4.6)—Dissolve 61.26 g of potassium hydrogen phthalate in about 800 mL of water, adjust the pH to 4.6 with sodium hydroxide TS, and add water to make 1000 mL.

(4) ***Dimethylaniline***—Weigh accurately about 1.0 g of Bacampicillin Hydrochloride, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Bacampicillin Hydrochloride taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 1.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 1.5 % (1 g)

Assay Weigh accurately about 40 mg (potency) each of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride RS, dissolve each in water to make exactly 100 mL, and use these solutions as the test solution and the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of bacampicillin of these solutions.

$$\text{Amount [μg (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = \text{Amount [μg (potency) of ampicillin] of Bacampicillin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: To 500 mL of 0.02 mol/L sodium dihydrogen phosphate, add 0.02 mol/L sodium hydrogen phosphate to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of bacampicillin is not less than 3000.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the operating conditions, the relative standard deviation of the peak areas of bacampicillin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Bacampicillin Hydrochloride Tablets

Bacampicillin Hydrochloride Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Tablets, with Bacampicillin Hydrochloride.

Identification Dissolve an amount of Bacampicillin Hydrochloride Tablets, equivalent to a suitable amount of bacampicillin hydrochloride according to the labeled amount, and a suitable amount of Bacampicillin Hydrochloride RS in ethanol (95) so that each mL contains 2 mg, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methylene chloride, chloroform, and ethanol (10 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.3 % ninhydrin-methanol solution on the plate: the spots obtained from the test solution and standard solution show the same R_f value.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Bacampicillin Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 56 mg of ampicillin ($C_{16}H_{19}N_3O_4S$), dissolve in water to make exactly 100 mL, and use this solution as the test solution. Weigh accurately about 80 mg (potency) of Bacampicillin Hydrochloride RS, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions and determine the peak areas, A_T and A_S, of bacampicillin.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) = Amount [μg (potency)] of Bacampicillin Hydrochloride RS $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 500 mL of 0.02 mol/L sodium dihydrogen phosphate, add 0.02 mol/L sodium hydrogen phosphate to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: 1 mL/minute

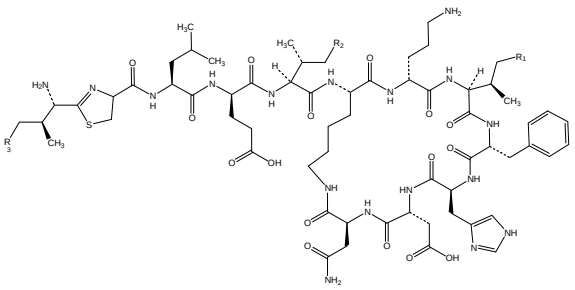
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of bacampicillin is not less than 3000.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bacampicillin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Bacitracin



	R_1	R_2	R_3
Bacitracin A:	CH_3	CH_3	CH_3
Bacitracin B_1:	CH_3	CH_3	H
Bacitracin B_2:	H	CH_3	CH_3
Bacitracin B_3:	CH_3	H	CH_3

[*1405-87-4*]

Bacitracin is a mixture of peptide substances having antibacterial activity including bacitracin A as the main component produced by the growth of *Bacillus subtilis* or *Bacillus licheniformis*.

Bacitracin contains not less than 60 units (potency) per mg of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$: 1422.69), calculated on the dried basis.

Description Bacitracin is white to light brown powder.

Bacitracin is freely soluble in water and slightly soluble in ethanol (99.5).

Identification (1) To 3 mL of a solution of Bacitracin (1 in 100) add 3 mL of 4-dimethylaminobenzaldehyde TS, shake until a deep red

to red-purple color appears, then add several drops of a solution of sodium nitrite (1 in 100), and shake: a green to dark green color is produced.

(2) Dissolve 60 mg each of Bacitracin and Bacitracin RS in 10 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine, and ethanol (99.5) (30 : 15 : 10 : 6 : 5) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 110 °C for 5 minutes: the spots obtained from the test solution and standard solution show the same R_f value.

pH The pH of a solution obtained by dissolving 1 g of Bacitracin in 10 mL of water is between 5.5 and 7.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Bacitracin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.15 g of Bacitracin in 0.05 mol/L sulfuric acid TS to make 100 mL. To 2 mL of this solution add 0.05 mol/L sulfuric acid TS to make 10 mL, and use this solution as the test solution. Determine the absorbances of this solution, A_1 and A_2, at 252 nm and 290 nm as directed under Ultraviolet-visible Spectrophotometry: A_2/A_1 is not more than 0.20.

Loss on Drying Not more than 5.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 1.0 % (1 g)

Sterility Test It meets the requirement, when Bacitracin is used in a sterile preparation.

Bacterial Endotoxins Less than 0.01 EU/mg (potency) of bacitracin, when Bacitracin is used in a sterile preparation.

Content Ratio of Bacitracin Weigh accurately a suitable amount of Bacitracin, dissolve in the mobile phase so that each mL contains 2.0 mg, and use this solution as the test solution. Perform the test with 100 μL each of the mobile phase, test solution, and quantitation limit solution as directed in the Content Ratio of Bacitracin under Bacitracin Zinc. The amount of bacitracin A is not less than 40.0 % and the amount of active bacitracin (bacitracin A, B_1, B_2, and B_3) is not less than 70.0 %. The amount of all peaks eluted before the peak of bacitracin B_1 (early eluting peptides) is not more than 20.0 %, and bacitracin F is not more than 6.0 %. Disregard any peak of the sample solution with an area smaller than the peak area of bacitracin A obtained from the quantitation limit solution, and any peak obtained from the mobile phase.

Assay ***The Cylinder-plate method*** (1) Culture medium Agar media for seed and base layer- Use the medium in I 2 1) (6) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Micrococcus luteus* ATCC 10240.

(3) Weigh accurately about 400 units (potency) of Bacitracin, and dissolve and dilute in 1 % phosphate buffer solution, pH 6.0 to make the solution so that each mL contains 2.0 and 0.5 units (potency) and use these solutions as the high concentration test solution and low concentration test solution. Separately, weigh accurately about 400 units (potency) of Bacitracin RS, and dissolve in 1 % phosphate buffer solution, pH 6.0 to make the solution so that each mL contains 5 units (potency), and use this solution as the standard stock solution. Keep the stock standard solution at not exceeding 10 °C and use within 2 days. Take exactly a suitable amount of the stock standard solution before use, add 1 % phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2.0 and 0.5 units (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—In a cold place.

Bacitracin Ointment

Bacitracin Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$: 1421.77).

Method of Preparation Prepare as directed under Ointments, with Bacitracin.

Identification Weigh about 5 g of Bacitracin Ointment and transfer to a beaker. Wash with two to three 20 mL volumes of chloroform, discard the washings, dissolve in a small amount of ethanol (95), and evaporate to dryness. Proceed with the residue as directed in the Identification under Bacitracin.

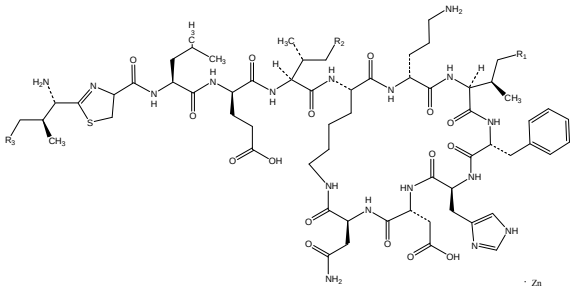
Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration)

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Bacitracin. Weigh accurately an amount of Bacitracin Ointment, equivalent to 500 to 2000 units (potency) according to the labeled potency, transfer to a separatory funnel, add 50 mL of ether, and shake until homogeneous. Extract with three 25 mL volumes of 1 % phosphate buffer solution (pH 6.0),

combine the extracts, and add 1 % phosphate buffer solution (pH 6.0) to make 100 mL. Pipet a suitable amount of this solution, dilute with 1 % phosphate buffer solution (pH 6.0) to make the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Bacitracin Zinc



	R_1	R_2	R_3
Bacitracin A:	CH_3	CH_3	CH_3
Bacitracin B_1:	CH_3	CH_3	H
Bacitracin B_2:	H	CH_3	CH_3
Bacitracin B_3:	CH_3	H	CH_3

[*1405-89-6*]

Bacitracin Zinc contains not less than 65 units (potency) per mg of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$: 1422.69), calculated on the dried basis. Bacitracin Zinc also contains not less than 4.0 % and not more than 6.0 % of zinc (Zn: 65.41), calculated on the dried basis

Description Bacitracin Zinc is white to pale yellow powder, is odorless or a tint of odor, and has a bitter taste.
Bacitracin Zinc is slightly soluble in water or in ethanol, very slightly soluble in ether, and practically insoluble in chloroform.
Bacitracin Zinc is hygroscopic.

Identification Dissolve a suitable amount of Bacitracin Zinc in 0.1 mol/L hydrochloric acid to make a solution containing 500 units per mL, and use this solution as the test solution. Separately, dissolve a suitable amount of Bacitracin Zinc RS to make a solution containing 500 units per mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, 2-propanol, dichloromethane, ammonium hydroxide, and water (4:2:2:1:5) to a distance of about 15 cm, and dry the plate at 105 °C for 10 minutes. Spray evenly 0.2 % ninhydrin-butanol solution on the plate and heat at 105 °C for 5 minutes: the spots obtained from the test solution and standard solution are dark purple in color and show the same R_f value.

pH The pH of a saturated solution of Bacitracin Zinc is between 6.0 and 7.5.

Loss on Drying Not more than 5.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Bacitracin Zinc is used in a sterile preparation.

Content Ratio of Bacitracin Zinc Weigh accurately a suitable amount of Bacitracin Zinc, dissolve in the diluent to make a solution containing 2.0 mg per mL, and use this solution as the test solution. Perform the test with 100 μL each of the diluent, test solution, and quantitation limit solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of bacitracin A according to the following equation (1) and the amount of active bacitracin (bacitracin A, B_1, B_2, and B_3) according to the equation (2): not less than 40.0 % and not less than 70.0 %, respectively. Calculate the amount of all peaks eluted before the peak of bacitracin B_1 (early eluting peptides) according to the equation (3) and the amount of bacitracin F according to the equation (4): not more than 20.0 % and not more than 6.0 %, respectively. Diregard any peak obtained from the test solution with an area smaller than the peak area of bacitracin A obtained from the quantiation limit solution, and any peak obtained from the diluent.

$$\text{Amount (\%) of bacitracin A} = \frac{A_A}{A_T} \times 100 \quad (1)$$

A_A: Peak area of bacitracin A obtained from the test solution
A_T: Total area of all peaks obtained from the test solution

$$\text{Amount (\%) of active bacitracin} = \frac{A_A + A_{B1} + A_{B2} + A_{B3}}{A_T} \times 100 \quad (2)$$

A_A, A_{B1}, A_{B2}, A_{B3}: Each peak area of bacitracin A, B_1, B_2, and B_3
A_T: Total area of all peaks obtained from the test solution

$$\text{Amount (\%) of early eluting peptides} = \frac{A_i}{A_T} \times 100 \quad (3)$$

A_i: Total area of all peaks eluted before the peak of bacitracin B_1
A_T: Total area of all peaks obtained from the test solution

$$\text{Amount (\%) of bacitracin F} = \frac{A_F}{A_A} \times 100 \quad (4)$$

A_F: Peak area of bacitracin F
A_A: Peak area of bacitracin A

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of methanol, water, phosphate buffer solution (pH 6.0), and acetonitrile (26 : 15 : 5 : 2)

Flow rate: 1.0 mL/minute

System suitability

System performance: Proceed with 100 μL of the solution for peak identification under the above operating conditions at 300 nm, and identify the location of the peak of bacitracin F with a relative retention time of about 2.4. Change the wavelength to 254 nm, proceed with 100 μL of the system suitability solution under the above operating conditions, and identify the peaks of the active components of bacitracin (bacitracin A, B_1, B_2, and B_3), early eluting peptides eluted before bacitracin B_1, and bacitracin F by referring to the relative retention times. The relative retention times of bacitracin C_1, C_2, C_3, B_1, B_2, B_3, and F are about 0.5, 0.6, 0.6, 0.7, 0.7, 0.8, and 2.4, respectively. Calculate the peak-to-valley ratio (*H*v/*H*p) of the height above the baseline of the peak of bacitracin B_1 (*H*p) with respect to the height above the baseline of the point separating the peak of bacitracin B_1 from the peak of bacitracin B_2 (*H*v): not more than 1.2.

Time span of measurement: About 3 times as long as the retention time of bacitracin A

Diluent—Dissolve 40 g of disodium dihydrogen ethylenediaminetetraacetate in 1000 mL of water and adjust the pH to 7.0 with dilute sodium hydroxide TS.

System suitability solution—Weigh accurately a suitable amount of Bacitracin Zinc RS and dissolve in the mobile phase so that each mL contains about 2.0 mg.

Quantitation limit solution—Dilute the system suitability solution with water so that each mL contains about 0.01 mg.

Solution for peak identification—Heat the system suitability solution in a water bath for 30 minutes and cool to room temperature.

Phosphate buffer solution (pH 6.0)—Dissolve 34.8 g of potassium hydrogen phosphate in 1000 mL of water and adjust the pH of this solution to 6.0 with a solution prepared by dissolving 27.2 g of potassium dihydrogen phosphate in 1000 mL of water.

Assay (1) ***Bacitracin—The Standard curve method*** 1) Culture medium Agar media for seed and base layer- Use the medium in I 2 1) (6) ① under Microbial Assay for Antibiotics. .

2) Test organism- Use *Micrococcus luteus* ATCC 10240 as test organism.

3) Weigh accurately an appropriate amount of Bacitracin Zinc, and dissolve in 0.01 mol/L hydrochloric acid TS to make the solution so that each mL contains 100 units (potency), pipet an appropriate amount of this solution and dilute in 1 % phosphate buffer solution, pH 6.0 to make the solution so that each mL conains 1.00 units (potency), and use this solution as the test solution. Separately, weigh accurately an appropriate amount of Bacitracin RS, dissolve in 0.01 mol/L hydrochloric acid TS to make the solution so that each mL contains 100 units (potency), and use this solution as the standard stock solution. Pipet an appropriate amount of this standard stock solution and dilute in 1 % phosphate buffer solution, pH 6.0 to make the solutions so that each mL contains 0.64, 0.80, 1.00, 1.25, and 1.56 units (potency) and use these solutions as the standard solutions. Also use 1.0 unit (potency) per mL as the mid-diluted solution. Perform the test with these solutions according to the Standard curve method (II 4) as directed under Microbial Assay for Antibiotics.

(2) ***Zinc—***Weigh accurately about 0.2 g (potency) of Bacitracin Zinc, dissolve in 0.01 mol/L hydrochloric acid TS, and make the solution 100 mL. Pipet 2 mL of this solution and add 0.001 mol/L hydrochloric acid TS to make 200 mL and use this solution as the test solution. Separately, weigh accurately about 3.11 g of zinc oxide, add 80 mL of 1 mol/L hydrochloric acid TS, heat to dissolve, allow to cool down to room temperature, and add water to make exactly 250 mL. Pipet an appropriate amount of this solution, dilute 0.001 mol/L hydrochloric acid TS to make the solutions contain 0.5, 1.5 and 2.5 μg of zinc per mL, and use these solutions as the standard solutions. Perform the test with the test solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry. The operating wavelength is 213.8 nm. Determine absorbances and calculate the amount of zinc according to the calibration curve method. The blank test solution is 0.001 mol/L hydrochloric acid TS.

$$\text{Amount [\%] of zinc} = \frac{C \times 100000}{W \times (100 - m)}$$

C: Amount [μg /mL] of zinc in the test solution
W: Amount [mg] of Bacitracin Zinc taken
m: loss on drying (%)

Containers and Storage ***Containers***—Tight containers.

Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Ointment

Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of bacitracin, neomycin ($C_{23}H_{46}N_6O_{13}$: 614.65), and polymyxin B.

Method of Preparation Prepare as directed under Ophthalmic Ointments, with Bacitracin Zinc, Neomycin Sulfate, and Polymyxin B Sulfate.

Identification (1) ***Bacitracin zinc***—Proceed as directed in the Identification under Bacitracin Zinc.

(2) ***Neomycin sulfate and polymyxin B sulfate***—Proceed as directed in the Identification under Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ointment.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Test for Metal Particles It meets the requirement.

Assay (1) ***Bacitracin zinc—The Standard curve method*** Proceed as directed in the Assay (1) under Bacitracin Zinc. Weigh accurately an amount of Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Ointment, equivalent to about 1000 units (potency) according to the labeled potency of batracin zinc, and transfer to a separatory funnel. Add 50 mL of ether, shake, add 20 to 25 mL of 0.01 mol/L hydrochloric acid TS, shake well, and extract. Take the hydrochloric acid layer, extract again with three 20 to 25 mL volumes of 0.01 mol/L hydrochloric acid TS in the same manner, combine the hydrochloric acid layers, and add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet a suitable amount of this solution, dilute with 1 % phosphate buffer solution (pH 6.0) so that each mL contains 1.00 units (potency), and use this solution as the test solution. If the concentration of bacitracin dissolved in 0.01 mol/L hydrochloric acid TS is not more than 1.00 units (potency) per mL, add to the dilution concentration for the standard curve of the standard solution the same amount of 0.01 mol/L hydrochloric acid TS as the amount of hydrochloric acid in the test solution. Separately, weigh accurately a suitable amount of Bacitracin RS and dissolve in 0.01 mol/L hydrochloric acid TS to make a standard stock solution containing 10 units (potency) per mL. Pipet a suitable amount of the standard stock solution, dilute with 1 % phosphate buffer solution (pH 6.0) so that each mL contains 0.64, 0.80, 1.00, 1.25, and 1.56 units (potency) per mL, use these solutions as the standard solutions, and use the solution containing 1.00 units (potency) per mL as the standard mid-diluted solution. Perform the test with the test solution, standard solutions, and standard mid-diluted solution as directed in II) (4) under Microbial Assay for Antibiotics.

(2) ***Neomycin sulfate—The Cylinder-plate method*** Proceed as directed in the Assay (2) under Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ointment.

(3) ***Polymyxin B sulfate—The Cylinder-plate method*** Proceed as directed in the Assay (3) under Bacitracin Zinc·Neomycin Sulfate·Polymyxin B Sulfate Ointment.

Containers and Storage ***Containers***—Tight containers.

Bacitracin·Neomycin Sulfate·Polymyxin B Sulfate Ointment

Bacitracin·Neomycin Sulfate·Polymyxin B Sulfate Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of bacitracin, neomycin ($C_{23}H_{46}N_6O_{13}$: 614.65), and polymyxin B.

Method of Preparation Prepare as directed under Ointments, with Bacitracin, Neomycin Sulfate, and Polymyxin B Sulfate.

Identification Dissolve suitable amounts of Bacitracin·Neomycin Sulfate·Polymyxin B Sulfate Ointment and each RS in 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1 mg (potency) or 200 units (potency), and use these solutions as the test solution and standard solutions. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot the test solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), and water (60 : 20 : 20), and air-dry the plate. Spray evenly a 1 % solution of ninhydrin in butanol containing a small amount of pyridine on the plate: the spots obtained from the test solution and standard solutions show the same R_f value.

Water Not more than 1.0 % (1 g, volumetric titration, direct titration)

Assay (1) ***Bacitracin—The Cylinder-plate method*** Proceed as directed in the Assay under Bacitracin Ointment.

(2) ***Neomycin—The Cylinder-plate method*** Proceed as directed in the Assay under Neomycin Sulfate. Weigh accurately an amount of Bacitracin·Neomycin Sulfate·Polymyxin B Sulfate Ointment, equivalent to

about 10 mg (potency) according to the labeled potency of neomycin, and transfer to a separatory funnel. Add 100 mL of ether, shake, extract with 50 mL of 0.1 mol/L phosphate buffer solution (pH 8.0) by mixing well, and take the buffer layer. Repeat this procedure twice with 20 mL each of 0.1 mol/L phosphate buffer solution (pH 8.0), combine the buffer layers, shake with 50 mL of butanol, and discard the butanol layer. Repeat this procedure twice with 25 mL each of butanol, and discard the butanol layer. To the buffer layer add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer solution (pH 8.0) to make the concentration of (3), and use this solution as the test solution.

(3) ***Polymyxin B sulfate—The Cylinder-plate method*** (1) Culture medium

(i) Agar medium for seed layer-

Pancreatic digest of casein	17.0 g
Polysorbate 80	10.0 g
Sodium chloride	5.0 g
Potassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Papaic digest of soybean meal	3.0 g
Agar	13.0 ~ 20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with 1 mol/L sodium hydroxide TS so that it will be 7.2 to 7.3 after sterilization.

(ii) Agar medium for base layer-

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Potassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Agar	13.0 ~ 20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with 1 mol/L sodium hydroxide TS so that it will be 7.2 to 7.3 after sterilization.

(iii) Agar medium for transferring test organism-

Peptone	6.0 g
Meat extract	1.5 g
Pancreatic digest of casein	4.0 g
Glucose	1.0 g
Yeast extract	3.0 g
Agar	13.0 ~ 20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with 1 mol/L sodium hydroxide TS so that it will be 6.5 to 6.6 after sterilization.

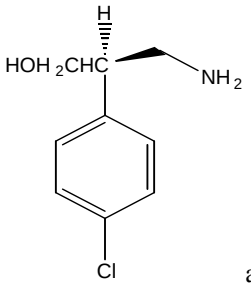
(2) Test organism- *Bordetella bronchiseptica* ATCC 4617.

(3) Test organism suspension- Incubate the test organism on the agar medium for transferring test organism at 32 to 37 °C for 16 to 24 hours, and subculture at least three times. Inoculate the subcultured test organism onto the slant of the agar medium for transferring test organism, incubate at 32 to 37 °C for 16 to 24 hours, and suspend in a suitable amount of sterile purified water. Use a photoelectric photometer to make the transmittance at 660 nm of this suspension 60 %. Store the suspension at a temperature not exceeding 15 °C and use within 3 days. To 100 mL of the agar medium for the seed layer, previously melted and cooled to 48 °C, add 0.13 mL of this adjusted suspension and use as the test organism suspension.

(4) Weigh accurately an amount of Bacitracin·Neomycin Sulfate·Polymyxin B Sulfate Ointment, equivalent to about 8000 to 20000 units (potency) according to the labeled potency of polylmyxin B sulfate, and transfer to a separatory funnel. Add 20 mL of ether, shake, and extract with 20 mL of 10 % phosphate buffer solution (pH 6.0). Repeat the extraction with two 10 mL volumes of 10 % phosphate buffer solution (pH 6.0), combine the buffer layers, and add 10 % phosphate buffer solution (pH 6.0) to make exactly 50 mL. Pipet a suitable amount of this solution, dilute with 10 % phosphate buffer solution (pH 6.0) so that each mL contains 100.0 and 25.0 units (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 20000 to 30000 units (potency) of Polymyxin B RS, and dissolve in 10 % phosphate buffer solution (pH 6.0) to make a standard stock solution containing 1000 units (potency) per mL. Pipet a suitable amount of the standard stock solution, dilute with 10 % phosphate buffer solution (pH 6.0) so that each mL contains 100.0 and 25.0 units (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I) (8) under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Preserve in tight containers.

Baclofen



and enantiomer

$C_{10}H_{12}ClNO_2$: 213.66

(*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid
[*1134-47-0*]

Baclofen contains not less than 98.5 % and not more than 101.0 % of baclofen ($C_{10}H_{12}ClNO_2$), calculated on the anhydrous basis.

Description Baclofen is a white to pale yellowish white, crystalline powder.
Baclofen is freely soluble in acetic acid (100), slightly soluble in water, very slightly soluble in methanol or in ethanol (95) and practically insoluble in ether.
Baclofen dissolves in dilute hydrochloric acid.

Identification (1) To 5 mL of a solution of Baclofen (1 in 1000), add 1 mL of ninhydrin TS and heat in a water-bath for 3 minutes: a blue-purple color develops.

(2) Determine the absorption spectra of solutions of Baclofen and Baclofen RS in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Baclofen as directed under the Flame Coloration Test (2): a green color appears.

Purity (1) ***Chloride***—Dissolve 0.5 g of Baclofen in 50 mL of acetic acid (100) and add water to make 100 mL. To 100 mL of this solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.21 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Baclofen according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Baclofen according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Baclofen in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1.0 mL and 1.5 mL of the test solution, to each add the mobile phase to make exactly 100 mL and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with 25 μL each of the test solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak height of these solutions: each height of the peaks other than the peak of baclofen from the test solution is not larger than the peak height of baclofen from the standard solution (1) and the total height of these peaks is not larger than the peak height of baclofen from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 268 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 900) (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of baclofen is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of baclofen after the solvent peak.

System suitability

Test for required detection: Adjust the sensitivity so that the peak height of baclofen obtained from 25 μL of the standard solution (1) is between 5 mm and 10 mm.

System performance: Dissolve 0.40 g of Baclofen and 5 mg of methyl parahydroxybenzoate in 200 mL of the mobile phase. To 10 mL of this solution, add the mobile phase to make 100 mL. When the procedure is run with 25 μL of this solution under the above operating conditions, baclofen and methyl parahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak heights of baclofen is not more than 3.0 %.

Water Not more than 1.0 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.3 % (1 g).

Assay Weigh accurately about 0.5 g of Baclofen, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.366 mg of $C_{10}H_{12}ClNO_2$

Containers and Storage ***Containers***—Well-closed containers.

Baclofen Tablets

Baclofen Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$: 213.66).

Method of Preparation Prepare as directed under Tablets, with Baclofen.

Identification (1) To a portion of powdered Baclofen Tablets, equivalent to 10 mg of Baclofen according to the labeled amount, add 10 mL of water, shake well and filter. To 5 mL of the filtrate, add 1 mL of ninhydrin TS and proceed as directed in the Identification (1) under Baclofen.

(2) To a portion of powdered Baclofen Tablets, equivalent to 25 mg of Baclofen according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric

acid TS, shake for 15 minutes and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 257 nm and 261 nm, between 264 nm and 268 nm and between 272 nm and 276 nm.

(3) To a portion of powdered Baclofen Tablets, equivalent to 10 mg of Baclofen according to the labeled amount, add 2 mL of a mixture of methanol and acetic acid (100) (4 : 1), shake well, centrifuge and use the clear supernatant liquid as the test solution. Separately, dissolve 10 mg of Baclofen RS in 2 mL of a mixture of methanol and acetic acid (100) (4 : 1) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution and that from the standard solution show the same R_f value.

Dissolution Test Perform the test with 1 tablet of Baclofen Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 500 mL of water as the dissolution solution. Take 20 mL or more of the dissolve solution 45 minutes after starting the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent, filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of baclofen ($C_{10}H_{12}ClNO_2$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 10 mg of Baclofen RS (separately determined the water content), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 220 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate (%) of Baclofen Tablets in 45 minutes is not less than 70 %.

Dissolution rate (%) with respect to the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$) = $W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 50$

W_S: Amount (mg) of Baclofen RS, calculated on the anhydrous basis

C: Labeled amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$) in 1 tablet.

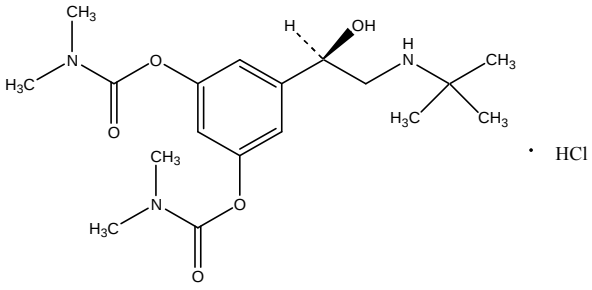
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Baclofen Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of baclofen ($C_{10}H_{12}ClNO_2$), add 130 mL of 0.1 mol/L hydrochloric acid TS, shake for 10 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL and centrifuge. Pipet 10 mL of the clear supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 0.25 g of Baclofen RS (separately determined the water content) and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL and use this solution as the standard solution. Pipet 2 mL each of the test solution and the standard solution, to each add 4 mL of ninhydrin-tin (II) chloride TS, shake, heat in a water-bath for 20 minutes and shake at once vigorously for 2 minutes. After cooling, to each solution, add a mixture of water and 1-propanol (1 : 1) to make exactly 25 mL. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 570 nm as directed under Ultraviolet-visible Spectrophotometry, using a blank prepared with 2 mL of water in the same manner.

Amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$) = Amount (mg) of Baclofen RS, calculated on the anhydrous basis × $\frac{A_T}{A_S}$

Containers and Storage ***Containers***—Well-closed containers.

Bambuterol Hydrochloride



and enantiomer

$C_{18}H_{29}N_3O_5$,HCl: 403.90

(*RS*)-[3-[2-(*tert*-Butylamino)-1-hydroxyethyl]-5-(dimethylcarbamoyloxy)phenyl] *N,N*-dimethylcarbamate hydrochloride [*81732-46-9*]

Bambuterol Hydrochloride contains not less than 98.5 % and not more than 101.5 % of bambuterol hydrochloride ($C_{18}H_{29}N_3O_5$,HCl), calculated on the anhydrous basis.

Description Bambuterol Hydrochloride is a white, crystalline powder.
Bambuterol Hydrochloride is freely soluble in water, and soluble in ethanol (95).
Bambuterol Hydrochloride shows polymorphism.

Identification (1) Determine the infrared spectra of Bambuterol Hydrochloride and Bambuterol Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Bambuterol Hydrochloride and Bambuterol Hydrochloride RS in a mixture of acetone and water (6 : 1), cool in ice to precipitate, then dry both precipitates in vacuum at 50 ºC to constant weight, and repeat the test with precipitates.

(2) The solution of Bambuterol Hydrochloride in water (1 in 50) responds to the Qualitative Tests for chloride (Method 2).

Specific Optical Rotation $[\alpha]_D^{20}$: −0.10 ~ +0.01° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity (1) ***Acidity or alkalinity*** —Dissolve 4.0 g of Bambuterol Hydrochloride in water to make exactly 20 mL. To 10 mL of the solution, add 0.2 mL of methyl red TS and 0.2 mL of 0.01 mol/L hydrochloric acid. The solution is red. When 0.4 mL of 0.01 mol/L sodium hydroxide is added, the solution becomes yellow.

(2) ***Related substances***—Dissolve 5.0 mg of Bambuterol Hydrochloride in the mobile phase, dilute to 10.0 mL with the mobile phase, and use this solution as the test solution. Separately, dissolve 1.0 mg of Formoterol Fumarate Dihydrate RS in the mobile phase, dilute to 10 mL with the mobile phase. Mix 0.8 mL of this solution with 0.4 mL of the test solution, dilute to 100 mL with the mobile phase, and use this solution as the standard solution (1). Dilute 1.0 mL of the test solution with the mobile phase to 50 mL. Dilute 2.0 ml of the solution to 20 mL with the mobile phase, and use this solution as the standard solution (2). Use the mobile phase as the blank solution. Perform the test with 20 μL each of the blank solution, the test solution, and the standard solutions as directed under Liquid Chromatography according to the following conditions. The area of any peak, other than from the principal peak, from the test solution is not greater than the area of the principal peak from the standard solution (2) (not more than 0.2 %); the sum of the areas of all the peaks, other than from the principal peak, is not greater than 3 times the area of the principal peak from the standard solution (2) (not more than 0.6 %). Disregard any peak obtained with the blank solution and any peak with an area less than 0.25 times the area of the principal peak obtained with the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm)

Column: A stainless steel column about 4.6 mm internal diameter and 15 cm in length, packed with base-deactivated octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 1.3 g of sodium octanesulphonate in 430 mL of a mixture of methanol and acetonitrile (75:25). Mix this solution with 570 mL of phosphate buffer solution.

Flow rate: 1.5 mL/min.

System suitability

System performance: Adjust the sensitivity of the system so that the height of the principal peak from the standard solution (2) is about 50 % of the full scale of the recorder. When the procedure is run with 20 μL of the standard solution (1), the retention times for formoterol and bambuterol are about 7 and 9 minutes, respectively, with the resolution between the peaks of bambuterol and formoterol being not less than 5.0.

Time span of measurement: 1.5 times the retention time of bambuterol.

Phosphate buffer solution—Dissolve 6.90 g of sodium dihydrogen phosphate monohydrate in water to make 1000 mL, and adjust the pH to 3.0 with 5 % phosphoric acid.

Water Not more than 0.5 % (0.5 g, volumetric titration, direct titration).

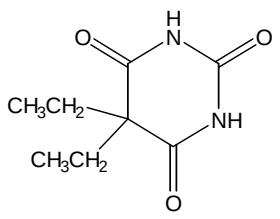
Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve 0.320 g of Bambuterol Hydrochloride, accurately weighed, in 50 mL of ethanol (95), add 5 mL of 0.01 mol/L hydrochloric acid, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Read the volume added between the two points of inflexion. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.39 mg of $C_{18}H_{30}ClN_3O_5$

Containers and Storage ***Containers***—Well-closed containers.

Barbital



$C_8H_{12}N_2O_3$: 184.19

5,5-Diethyl-1,3-diazinane-2,4,6-trione [*57-44-3*]

Barbital, when dried, contains not less than 99.0 % and not more than 101.0 % of barbital ($C_8H_{12}N_2O_3$).

Description Barbital appears as colorless or white crystals or crystalline powder, is odorless and has a slightly bitter taste.
Barbital is freely soluble in acetone or in pyridine, soluble in ethanol (95), sparingly soluble in ether and slightly soluble in water or in chloroform.
Barbital dissolve in sodium hydroxide TS and in ammonia TS.

pH—Its saturated solution is between 5.0 and 6.0.

Identification (1) Boil 0.2 g of Barbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 50 mg of Barbital in 5 mL of diluted pyridine (1 in 10), add 0.3 mL of copper (II) sulfate TS, shake and allow to stand for 5 minutes: a red-purple precipitate is formed. Shake the mixture with 5 mL of chloroform: a red-purple color develops in the chloroform layer. Separately, dissolve 50 mg of Barbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7 and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. The red-purple precipitate is not dissolve in the chloroform by shaking.

(3) To 0.4 g of Barbital, add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture in a water-bath under a reflux condenser for 30 minutes and allow to stand for 1 hour. Collect the separated crystals, wash with 7 mL of sodium hydroxide TS and a small amount of water, recrystalize from a mixture of ethanol (95) and chloroform (1 : 1) and dry at 105 ºC for 30 minutes: the crystals melt between 192 ºC and 196 ºC.

Melting Point 189 ~ 192 ºC

Purity (1) ***Clarity and color of solution***— Dissolve 0.5 g of Barbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.30 g of Barbital in 20 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid and add water to make 50 mL (not more than 0.035 %).

(3) ***Sulfate***—Dissolve 0.40 g of Barbital in 20 mL of acetone and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone and 1 mL of dilute hydrochloric acid and add water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Barbital according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Readily carbonizable substances***—Perform the test with 0.5 g of Barbital. The solution has not more color than Color Matching Fluid A.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.4 g of Barbital, previously dried and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through pale blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 18.419 mg of $C_8H_{12}N_2O_3$

Containers and Storage ***Containers***—Well-closed containers.

Barium Sulfate

$BaSO_4$: 233.39

Barium(2+) sulfate [*7727-43-7*]

Description Barium Sulfate is a white powder, is odorless and tasteless.
Barium Sulfate is practically insoluble in water, in ethanol (95) or in ether.
Barium Sulfate does not dissolve in hydrochloric acid, in nitric acid or in sodium hydroxide TS.

Identification (1) Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate in a crucible, heat the mixture until fusion is complete, treat the cooled mass with hot water and filter. The filtrate, acidified with hydrochloric acid, responds to the Qualitative Tests for sulfate.

(2) Wash the hot water-insoluble residue obtained in (1) with water, dissolve in 2 mL of acetic acid (31) and filter, if necessary: the solution responds to the Qualitative Tests for barium salt.

Purity (1) ***Acidity or alkalinity***—Agitate 1.0 g of Barium Sulfate with 20 mL of water for 5 minutes: the solution is neutral.

(2) ***Phosphate***—Boil 1.0 g of Barium Sulfate with 3 mL of nitric acid and 5 mL of water for 5 minutes,

cool and add water to restore the original volume. Filter through a filter paper, previously washed with dilute nitric acid, to the filtrate, add an equal volume of ammonium molybdate TS and allow to stand between 50 ºC and 60 ºC for 1 hour: no yellow precipitate is produced.

(3) ***Sulfide***—Place 10 g of Barium Sulfate in a 250 mL Erlenmeyer flask, add 10 mL of dilute hydrochloric acid and water to make 100 mL and boil for 10 minutes: the gas evolved does not darken moistened lead acetate paper.

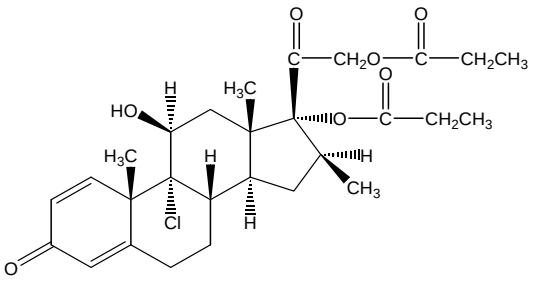
(4) ***Heavy metals***—Boil 5.0 g of Barium Sulfate with 2.5 mL of acetic acid (100) and 50 mL of water for 10 minutes, cool, add 0.5 mL of ammonia TS and water to make 100 mL and filter. Perform the test with 50 mL of this filtrate. Prepare the control solution with 2.5 mL of standard lead solution, 1.25 mL of acetic acid (100), 0.25 mL of ammonia TS and water to make 50 mL (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 2.0 g of Barium Sulfate according to Method 1 and perform the test (not more than 1 ppm).

(6) ***Hydrochloric acid-soluble substances and soluble barium salts***—Cool the solution obtained in (3), add water to make 100 mL and filter. Evaporate 50 mL of the filtrate on a water-bath to dryness, add 2 drops of hydrochloric acid and 10 mL of warm water, filter through filter paper for assay and wash with 10 mL of warm water. Evaporate the combined filtrate and washings on a water-bath to dryness and dry the residue at 105 ºC for 1 hour: the residue weighs not more than 15 mg. Shake the residue, if any, with 10 mL of water and filter. To the filtrate, add 0.5 mL of dilute sulfuric acid and allow to stand for 30 minutes: no turbidity is produced.

Containers and Storage ***Containers***—Well-closed containers.

Beclomethasone Dipropionate



$C_{28}H_{37}ClO_7$: 521.04

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-Chloro-11-hydroxy-10,13,16-trimethyl-3-oxo-17-[2-(propanoyloxy)ethanoyl]-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl propanoate [*5534-09-8*]

Beclomethasone Dipropionate, when dried, contains not less than 97.0 % and not more than 103.0 % of beclomethasone dipropionate ($C_{28}H_{37}ClO_7$).

Description Beclomethasone Dipropionate is a white to pale yellow powder and is odorless.
Beclomethasone Dipropionate is freely soluble in chloroform, soluble in methanol, sparingly soluble in ethanol (95) or in 1,4-dioxane, slightly soluble in ether and practically insoluble in water.

Melting point— About 208 ºC (with decomposition).

Identification (1) Dissolve 2 mg of Beclomethasone Dipropionate in 2 mL of sulfuric acid: initially a yellowish color develops and gradually changes through orange to dark red-brown. To this solution, add carefully 10 mL of water: the color changes to bluish green and a flocculent precipitate is formed.

(2) Dissolve 10 mg of Beclomethasone Dipropionate in 1 mL of methanol, add 1 mL of Fehling's TS and heat: a red to red-brown precipitate is formed.

(3) Prepare the test solution with 20 mg of Beclomethasone Dipropionate as directed under the Oxygen Flask Combustion Method, using a mixture of 1 mL of sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests for chloride.

(4) Determine the infrared spectra of Beclomethasone Dipropionate and Beclomethasone Dipropionate RS, previously dried, as directed in the potassium bromide disk method under the Infrared Spectro-photometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Beclomethasone Dipropionate and Beclomethasone Dipropionate RS in ethanol (95), then evaporate the ethanol (95) to dryness and repeat the test with the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +108° ~ +115° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Beclomethasone Dipropionate according to Method 2 and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm)

(2) ***Related substances***—Dissolve 20 mg of Beclomethasone Dipropionate in 5 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer

chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (475 : 25 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Asssy Weigh accurately, about 20 mg each of Beclomethasone Dipropionate and Beclomethasone Dipropionate RS, previously dried and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of the test solution and the standard solution, add exactly 10 mL of the internal standard solution and methanol to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Beclomethasone Dipropionate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of } C_{28}H_{37}ClO_7 = \text{Amount (mg) of Beclomethasone Dipropionate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of testosterone propionate in methanol (1 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of acetonitrile and water (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of Beclomethasone Dipropionate is about 6 minutes.

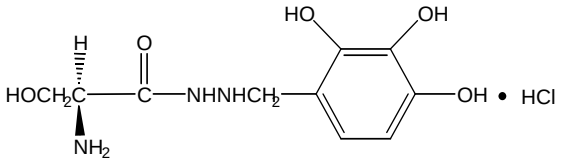
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, beclomethasone dipropionate and the internal standard are eluted in this order with the resolution between their peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of beclomethasone dipropionate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Benserazide Hydrochloride



and enantiomer

$C_{10}H_{15}N_3O_5 \cdot HCl$: 293.70

2-Amino-3-hydroxy-*N*′-[(2,3,4-trihydroxyphenyl)methyl] propanehydrazidehydrochloride [*14919-77-8*]

Benserazide Hydrochloride contains not less than 98.0 % and not more than 101.0 % of benserazide hydrochloride ($C_{10}H_{15}N_3O_5 \cdot HCl$), calculated on the anhydrous basis.

Description Benserazide Hydrochloride is a white to grayish white, crystalline powder.

Benserazide Hydrochloride is freely soluble in water or in formic acid, soluble in methanol, very slightly soluble in ethanol (95) and practically insoluble in ether.

Benserazide Hydrochloride is hygroscopic.

Benserazide Hydrochloride is gradually affected by light.

A solution of Benserazide Hydrochloride (1 in 100) shows no optical rotation.

pH—Dissolve 1.0 g of a solution of Benserazide Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.0.

Identification (1) Determine the absorption spectra of the solutions of Benserazide Hydrochloride and Benserazide Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Benserazide Hydrochloride and Benserazide Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry,: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) To 10 mL of a solution of Benserazide Hydrochloride (1 in 30), add silver nitrate TS: a white precipitate is produced. To a portion of this precipitate, add dilute nitric acid: the precipitate does not dissolve.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Benserazide Hydrochloride in 10 mL of water and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorb-

ance of this solution at 430 nm is not more than 0.10.

(2) ***Heavy metals***—Proceed with 1.0 g of Benserazide Hydrochloride in according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 0.25 g of Benserazide Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL and 3 mL of the test solution, add methanol to make exactly 200 mL and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solutions (1) and (2) on a plate of cellulose for thin-layer chromato-graphy. Develop the plate with a solution of formic acid in sodium chloride TS (1 in 1000) to a distance of about 10 cm and air-dry the plate. Spray evenly sodium carbonate TS, air-dry and then spray evenly Folin's TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (2) and the numbers of the spots which are more intense than the spot from the standard solution (1) are not more than 2.

Water Not more than 2.5 % (0.5 g, volumetric titration, direct titration). Use a solution of salicylic acid in methanol for Karl Fischer Method (3 in 20) instead of methanol for Karl Fischer Method.

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.3 g of Benserazide Hydrochloride, dissolve in 5 mL of formic acid, add 50 mL of acetic acid (100) and titrate immediately with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.370 mg of $C_{10}H_{15}N_3O_5 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Benzalkonium Chloride

Benzalkonium Chloride is represented by the formula $[C_6H_5CH_2N(CH_3)_2R]Cl$, in which R extends from C_8H_{17} to $C_{18}H_{37}$, with $C_{12}H_{25}$ and $C_{14}H_{29}$ comprising the major portion.

Benzalkonium Chloride contains not less than 95.0 % and not more than 105.0 % of benzalkonium chloride ($C_{22}H_{40}ClN$: 354.01) calculated on the anhydrous basis.

Description Benzalkonium Chloride is a white to yellowish white powder, colorless to pale yellow, gelatinous pieces or jelly-like fluid or mass. Benzalkonium Chloride has a characteristic odor.

Benzalkonium Chloride is very soluble in water or in ethanol (95) and practically insoluble in ether.

A solution of Benzalkonium Chloride foams strongly when shaken.

Identification (1) Dissolve 0.2 g of Benzalkonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate and heat for 5 minutes in a water-bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool and filter: the filtrate responds to the Qualitative Tests for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride (1 in 1000), add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color is observed. Add 4 mL of chloroform to this solution and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectra of the solutions of Benzalkonium Chloride and Benzalkonium Chloride RS in 0.1 mol/L hydrochloric acid VS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensitiesof absorption at the same wavenumbers.

(4) To 1 mL of a solution of Benzalkonium Chloride (1 in 100), add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Benzalkonium Chloride in 10 mL of water: the solution is clear and colorless, or pale yellow.

(2) ***Petroleum ether-soluble substances***—To 3.0 g of Benzalkonium Chloride, add water to make 50 mL, then add 50 mL of dehydrated ethanol and 5 mL of 0.5 mol/L sodium hydroxide TS and extract with three 50 mL volumes of petroleum ether. Combine the petroleum ether extracts and wash with three 50 mL volumes of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper and wash the filter paper with two 10 mL volumes of petroleum ether. Evaporate the petroleum ether on a water-bath by heating and dry the residue at 105 ℃ for 1 hour: the residue is not more than 1.0 %.

Water Not more than 15.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately 0.15 g of Benzalkonium

Chloride and dissolve in 75 mL of water. Adjust the pH between 2.6 and 3.4 by adding drop-wise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methylorange TS and titrate with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and Storage ***Containers***—Tight containers.

Benzalkonium Chloride Concentrated Solution 50

Benzalkonium Chloride Concentrated Solution 50 is an aqueous solution, presented as $[C_6H_5CH_2N(CH_3)_2R]$ Cl, where R ranges from C_8H_{17} to $C_{18}H_{37}$ and mainly consisting of $C_{12}H_{25}$ and $C_{14}H_{29}$.
Benzalkonium Chloride Concentrated Solution 50 contains not less than 50.0 % and not more than 55.0 % of benzalkonium chloride ($C_{22}H_{40}ClN$: 354.01).

Description Benzalkonium Chloride Concentrated Solution 50 is a clear, colorless to pale yellow liquid or jelly-like fluid and has a characteristic odor.
Benzalkonium Chloride Concentrated Solution 50 is very soluble in water or in ethanol (95) and practically insoluble in ether.
A solution prepared by adding water to Benzalkonium Chloride Concentrated Solution 50 vigorously foams when shaken.

Identification (1) Dissolve 0.4 g of Benzalkonium Chloride Concentrated Solution 50 in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 500), add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue cool shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectra of the solutions of Benzalkonium Chloride Concentrated Solution 50 and Benzalkonium Chloride RS in 0.1 mol/L hydrochloric acid VS (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 50), add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS; a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity (1) ***Clarity and color of solution***— Dissolve 2.0 g of Benzalkonium Chloride Concentrated Solution 50 in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Petroleum ether-soluble substances***—To 6.0 g of Benzalkonium Chloride Concentrated Solution 50, add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS and extract with three 50 mL volumes of petroleum ether. Combine the petroleum ether extracts and wash with three 50 mL volumes of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper and wash the filter paper with two 10 mL volumes of petroleum ether. Evaporate the petroleum ether on a water-bath by heating and dry the residue at 105 ºC for 1 hour: the residue is not more than 1.0 %.

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately 0.30 g of Benzalkonium Chloride Concentrated Solution 50 and dissolve in 75 mL of water. Adjust the pH between 2.6 and 3.4 by adding drop-wise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methylorange TS and titrate with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and Storage ***Containers***—Tight containers.

Benzalkonium Chloride Solution

Benzalkonium Chloride Solution is an aqueous solution containing not more than 50.0 w/v % of Benzalkonium Chloride.
Benzalkonium Chloride Solution contains not less than 93.0 % and not more than 107.0 % of the labeled amount of benzalkonium chloride ($C_{22}H_{40}ClN$: 354.01).

Method of Preparation Dissolve Benzalkonium Chloride in Water or Purified Water. Benzalkonium Chloride Solution is also prepared by diluting Benzalkonium Chloride Concentrated Solution 50 with Water or Purified Water.

Description Benzalkonium Chloride Solution is a clear, colorless to pale yellow liquid, and has a characteristic odor.
Benzalkonium Chloride Solution foams strongly on shaking.

Identification (1) Evaporate a volume of Benzalkonium Chloride Solution, equivalent to 0.2 g of Benzalkonium Chloride according to the labeled amount, on a water-bath to dryness and proceed with the residue as directed in the Identification (1) under Benzalkonium Chloride.

(2) To a volume of Benzalkonium Chloride Solution, equivalent to 10 mg of Benozalkonium Chloride according to the labeled amount, add water to make 10 mL. Proceed with 2 mL of this solution as directed in the Identification (2) under Benzalkonium Chloride.

(3) To a volume of Benzalkonium Chloride Solution, equivalent to 1 g of Benozalkonium Chloride according to the labeled amount, add water or concentrate on a water-bath, if necessary, to make 10 mL. To 1 mL of this solution, add 0.1 mol/L hydrochloric acid VS to make 200 mL and proceed as directed in the Identification (3) under Benzalkonium Chloride.

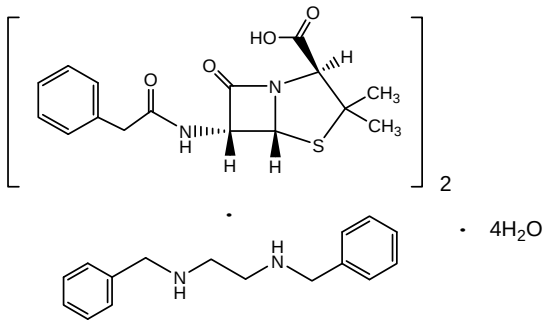
(4) To a volume of Benzalkonium Chloride Solution, equivalent to 0.1 g of Benozalkonium Chloride according to the labeled amount, add water or concentrate on a water-bath, if necessary, to make 10 mL. Proceed with 1 mL of this solution as directed in the Identification (4) under Benzalkonium Chloride.

Assay Pipet a volume of Benzalkonium Chloride Solution, equivalent to about 0.15 g of benzalkonium chloride ($C_{22}H_{40}ClN$), dilute with water to make 75 mL, if necessary and proceed as directed in the Assay under Benzalkonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and Storage ***Containers***—Tight containers.

Benzathine Penicillin G Hydrate



$(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2 \cdot 4H_2O$: 981.19

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzlethylenediamine(2:1), tetrahydrate [*41372-02-5*]

Benzathine Penicillin G Hydrate is the *N, N'*-dibenzylethylenediamine salt of a penicillin compound having antibacterial activity produced by the growth of *Penicillium* species.
Benzathine Penicillin G Hydrate contains not less than 1213 units (potency) and not more than 1333 units (potency) per mg of penicillin G sodium ($C_{16}H_{17}N_2NaO_4S$: 356.37), calculated on the anhydrous basis. One unit of Benzathine Penicillin G Hydrate is equivalent to 0.6 μg of penicillin G sodium. Benzathine Penicillin G Hydrate contains not less than 24.0 % and not more than 27.0 % of *N,N'*-dibenzylethylenediamine ($C_{16}H_{20}N_2$: 240.34), calculated on the anhydrous basis.

Description Benzathine Penicillin G Hydrate is a white crystalline powder.
Benzathine Penicillin G Hydrate is slightly soluble in methanol or in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Benzathine Penicillin G Hydrate and Benzathine Penicillin G RS in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Benzathine Penicillin G Hydrate and Benzathine Penicillin G RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Benzathine Penicillin G Weigh accurately about 50 mg of Benzathine Penicillin G Hydrate, dissolve in methanol and make exactly 100 mL. Determine absorbance of this solution at 263 nm with methanol as control solution, and obtain $E_{1\,cm}^{1\%}$.

$$\text{Amount [\%] of Benzathine Penicillin G} = E_{1\,cm}^{1\%} \times \frac{100}{7}$$

Specific Optical Rotation $[\alpha]_D^{20}$: +217 ~ +233° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm)

pH The pH of a saturated solution of Benzathine Penicillin G Hydrate is between 5.0 and 7.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Benzathine Penicillin G Hydrate according to Method

2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Benzathine Penicillin G Hydrate according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 70 mg of Benzathine Penicillin G Hydrate in 25 mL of methanol, add a solution prepared by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL to make 50 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution: the area of the peak having the relative retention time of about 2.4 with respect to penicillin G obtained from the test solution is not larger than 2 times the total area of the peaks of penicillin G and *N,N'*-dibenzylethylenediamine obtained from the standard solution, and the area of the peak other than penicillin G, *N,N'*-dibenzyl-ethylene-diamine and the peak having the relative retention time of about 2.4 with respect to benzylpenicillin obtained from the test solution is not larger than the total area of the peaks of penicillin G and *N,N'*-dibenzyl-ethylenediamine obtained from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water, methanol, and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (6 : 3 : 1)

Mobile phase B: A mixture of methanol, water, and 0.25 mol/L potassium dihydrogen phosphate (pH 3.5) (6 : 3 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	75	25
10-20	75→0	25→100
20-55	0	100

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add mobile phase A to make exactly 20 mL. Confirm that the peak area of penicillin G obtained from 20 μL of this solution is equivalent to 3.5 to 6.5 % of that from the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N,N'*-dibenzylethylenediamine and penicillin G are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 3 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak are of penicillin G is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of penicillin G beginning ater the solvent peak.

Water 5.0 ~ 8.0 % (1 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Benzathine Penicillin G Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.01 EU/100 units (potency) of penicillin G, when Benzathine Penicillin G Hydrate is used in a sterile preparation.

Assay (1) ***Penicillin G***—Weigh accurately an amount of Benzathine Penicillin G Hydrate, equivalent to about 85000 units, dissolve in 25 mL of methanol, and add a solution prepared by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of a solution prepared by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1 : 1) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Penicillin G Potassium RS, equivalent to about 85000 units, and about 25 mg of *N,N'*-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add a solution prepared by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of 50 mL of a solution prepared by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and 50 mL of methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of penicillin G.

$$\text{Amount (unit) of penicillin G} = \text{Amount (unit) of Penicillin G Potassium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: A ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of water, methanol, and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (11 : 17 : 2)

Flow rate: Adjust the flow rate so that the retention time of penicillin G is about 18 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N,N'*-dibenzylethylenediamine and penicillin G are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N,N'*-dibenzylethylenediamine and penicillin G are not more than 2.0 %, respectively.

(2) ***N,N'-Dibenzylethylenediamine***—Determine the areas, A_T and A_S, of the peak corresponding to *N,N'*-dibenzylethylenediamine on the chromatograms obtained in (1) with the test solution and standard solution.

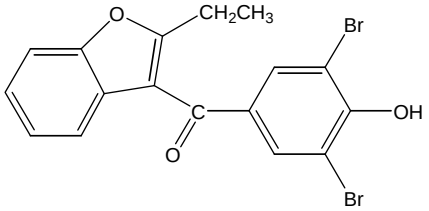
$$\text{Amount (\%) of } N,N'\text{-dibenzylethylenediamine } (C_{16}H_{20}N_2) = \frac{\text{Amount (mg) of } N,N'\text{-dibenzyle thylenedia mine diacetate taken}}{\text{Amount (mg) of Benzathine Penicillin G Hydrate taken}} \times \frac{A_T}{A_S} \times 100 \times 0.667$$

0.667: Conversion factor of the molecular mass of *N,N'*-dibenzylethylenediamine diacetate ($C_{16}H_{20}N_2 \cdot 2CH_3COOH$) to that of *N,N'*-dibenzylethylenediamine (benzathine, $C_{16}H_{20}N_2$)

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Benzbromarone



$C_{17}H_{12}Br_2O_3$: 424.08

(3,5-Dibromo-4-hydroxyphenyl)-(2-ethyl-1-benzofuran-3-yl)methanone [*3562-84-3*]

Benzbromarone, when dried, contains not less than 98.5 % and not more than 101.0 % of benzbromarone ($C_{17}H_{12}Br_2O_3$).

Description Benzbromarone is a white to pale yellow powder, is odorless and tasteless.

Benzbromarone is very soluble in *N,N*-dimethylformamide, freely soluble in acetone or in chloroform, soluble in ether, sparingly soluble in ethanol (95) and practically insoluble in water.

Benzbromarone dissolves in dilute sodium hydroxide TS.

Identification (1) Determine the absorption spectra of the solutions of Benzbromarone and Benzbromarone RS in 0.01 mol/L sodium hydroxide TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Benzbromarone and Benzbromarone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 149 ~ 153 ºC

Purity (1) ***Sulfate***—Dissolve 1.0 g of Benzbromarone in 40 mL of acetone and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 40 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019 %).

(2) ***Soluble halides***—Dissolve 0.5 g of Benzbromarone in 40 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Proceed with this solution as directed under the Chloride Limit Test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS, add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL.

(3) ***Heavy metals***—Proceed with 2.0 g of

Benzbromarone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Iron***—Prepare the test solution with 1.0 g of Benzbromarone according to Method 3 and perform the test according to Method A. Prepare the control solution with 2.0 mL of standard iron solution (not more than 20 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Benzbromarone in 10 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 4-methyl-2-pentanol, ethanol (99.5), and acetic acid (100) (100 : 20 : 2 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum at a pressure not exceeding 0.67 kPa, P_2O_5, 50 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

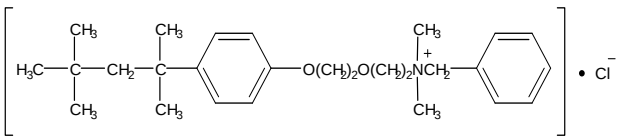
Assay Weigh accurately about 0.6 g of Benzbromarone, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 42.41 mg of $C_{17}H_{12}Br_2O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Benzethonium Chloride



$C_{27}H_{42}ClNO_2$: 448.08

Benzyl-dimethyl-[2-[2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethoxy]ethyl]azanium chloride [*121-54-0*]

Benzethonium Chloride, when dried, contains not less than 97.0 % and not more than 101.0 % of benzethonium chloride($C_{27}H_{42}ClNO_2$).

Description Benzethonium Chloride appears as colorless or white crystals and is odorless.
Benzethonium Chloride is very soluble in ethanol (95), freely soluble in water and practically insoluble in ether. A solution of Benzethonium Chloride foams strongly when shaken.

Identification (1) Dissolve 0.2 g of Benzethonium Chloride in 1 mL of sulfuric acid, add 0.1 g sodium nitrate and heat for 5 minutes in a water-bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool and filter: the filtrate responds to the Qualitative Tests for primary aromatic amines, with a red color observed.

(2) To 2 mL of a solution of Benzethonium Chloride (1 in 1000), add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color is observed. Add 4 mL of chloroform to this solution and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer and add dropwise a solution of sodium lauryl sulfate (1 in 1000) with stirring: the chloroform layer turns colorless.

(3) Determine the absorption spectra of the solutions of Benzethonium Chloride and Benzethonium Chloride RS in 0.1 mol/L hydrochloric acid VS (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzethonium Chloride (1 in 100), add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on addition of dilute nitric acid, but dissolves on addition of ammonia TS.

Melting Point 158 ~ 164 ºC (after drying).

Purity ***Ammonium***—Dissolve 0.1 g of Benzethonium Chloride in 5 mL of water, add 3 mL of sodium hydroxide and boil this solution. The wet red litmus paper does not change to blue in color by gas produced from the solution.

Loss on Drying Not more than 5.0 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.2 g of Benzethonium Chloride, previously dried, dissolve in 75 mL of diluted dilute hydrochloric acid (1 in 2) dropwise to adjust the pH 2.6 to 3.4, then add 1 drop of methyl orange TS and titrate with 0.02 mol/L sodium tetraphenylboron VS until the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Benzethonium Chloride Solution

Benzethonium Chloride Solution contains not less than 93.0 % and not more than 107.0 % of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$: 448.08).

Method of Preparation Dissolve Benzethonium Chloride in Water or Purified Water.

Description Benzethonium Chloride Solution is a clear, colorless liquid and is odorless.
Benzethonium Chloride Solution foams strongly when shaken.

Identification (1) Evaporate a volume of Benzethonium Chloride Solution, equivalent to 0.2 g of Benzethonium Chloride according to the labeled amount, on a water-bath to dryness and proceed with the residue as directed in the Identification (1) under Benzethonium Chloride.

(2) To a volume of Benzethonium Chloride Solution, equivalent to 10 mg of Benzethonium Chloride according to the labeled amount, add water to make 10 mL and proceed with 2 mL of this solution as directed in the Identification (2) under Benzethonium Chloride.

(3) To a volume of Benzethonium Chloride Solution, equivalent to 1 g of Benzethonium Chloride according to the labeled amount, add water or concentrate on a water-bath, if necessary, to make 10 mL. To 1 mL of this solution, add 0.1 mol/L hydrochloric acid VS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry : it exhibits maxima abetween 262 nm and 264 nm, between 268 nm and 270 nm, and between 274 nm and 276 nm.

(4) To a volume of Benzethonium Chloride Solution, equivalent to 0.1 g of Benzethonium Chloride according to the labeled amount, add water or concentrate on a water-bath, if necessary, to make 10 mL and proceed with 1 mL of this solution as directed in the Identification (4) under Benzethonium Chloride.

Purity (1) ***Nitrite***—Add 1.0 mL of Benzethonium Chloride Solution to a mixture of 1.0 mL of a solution of glycine (1 in 10) and 0.5 mL of dilute acetic acid: no gas is evolved.

(2) ***Oxidizing substances***—To 5 mL of Benzethonium Chloride Solution, add 0.5 mL of potassium iodide TS and 2 to 3 drops of dilute hydrochloric acid: no yellow color is observed.

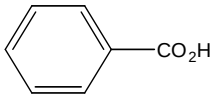
Assay Pipet a volume of Benzethonium Chloride Solution, equivalent to about 0.2 g of benzethonium chloride ($C_{27}H_{42}ClNO_2$), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzethonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Benzoic Acid



$C_7H_6O_2$: 122.12

[*65-85-0*]

Benzoic Acid, when dried, contains not less than 99.5 % and not more than 101.0 % of benzoic acid ($C_7H_6O_2$).

Description Benzoic Acid appears as white crystals or crystalline powder, is odorless, or has a faint, benzaldehyde-like odor.
Benzoic Acid is freely soluble in ethanol (95), in acetone, or in ether, soluble in hot water and slightly soluble in water.

Identification Dissolve 1 g of Benzoic Acid in 8 mL of sodium hydroxide TS and add water to make 100 mL: this solution responds to the Qualitative Tests (2) for benzoate.

Melting Point 121 ~ 124 ºC.

Purity (1) ***Heavy metals***—Dissolve 1.0 g of Benzoic Acid in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of standard lead solution, add 2 mL of dilute acetic acid, 25 mL of acetone and water to make 50 mL (not more than 10 ppm).

(2) ***Chlorinated compounds***—Take 0.5 g of Benzoic Acid and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water and dry. Ignite it at about 600 ºC, dissolve in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL and add 0.5 mL of silver nitrate TS: this solution has no more turbidity than the following control solution.

Control solution—Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL and add 0.5 mL of silver nitrate TS.

(3) ***Potassium permanganate-reducing substances***—Add 0.02 mol/L potassium permanganate VS drop-wise to a boiling mixture of 100 mL of water and 1.5 mL of sulfuric acid, until a red color persists for 30 seconds. Dissolve 1.0 g of Benzoic Acid in this boiling solution and add 0.50 mL of 0.02 mol/L potassium permanganate VS: a red color persists for at least 15 seconds.

(4) ***Phthalic acid***—Dissolve exactly about 100 mg of Benzoic Acid in 1 mL of water, add 1 mL of resorcinol-sulfuric acid TS, and heat in an oil bath at a temperature between 120 and 125 °C. After evaporating the water, heat again for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of this solution add 10 mL of a solution of sodium hydroxide (43 in 500), and use this solution as the test solution. Separately, dissolve exactly 61 mg of potassium biphthalate in 1000 mL of water. Proceed with 1 mL of this solution in the same manner as the test solution, and use this solution as the standard solution. Determine the absorbances at 495 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution is not more than that of the standard solution.

(5) ***Readily carbonizable substances***— Perform the test using 0.5 g of Benzoic Acid. The solution has no more color than Color Matching Fluid Q.

Loss on Drying Not more than 0.5 % (1 g, silica gel, 3 hours).

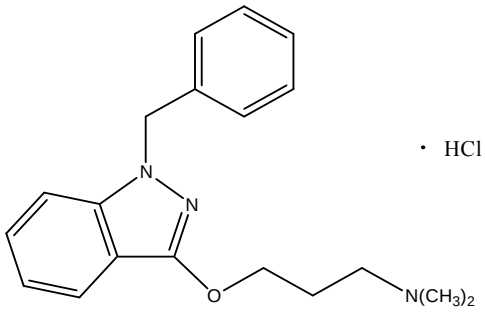
Residue on Ignition Not more than 0.05 % (1 g).

Assay Weigh accurately about 0.5 g of Benzoic Acid, previously dried, dissolve in 25 mL of neutralized ethanol and 25 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.212 mg of $C_7H_6O_2$

Containers and Storage ***Containers***—Well-closed containers.

Benzydamine Hydrochloride



$C_{19}H_{23}N_3O \cdot HCl$: 345.87

3-(1-Benzylindazol-3-yl)oxy-*N,N*-dimethylpropan-1-amine hydrochloride [*132-69-4*]

Benzydamine Hydrochloride contains not less than 99.0 % and not more than 101.0 % of benzydamine hydrochloride ($C_{19}H_{23}N_3O \cdot HCl$), calculated on the dried basis.

Description Benzydamine Hydrochloride is a white crystalline powder.
Benzydamine Hydrochloride is very soluble in water, freely soluble in ethanol (95) or in chloroform, and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Benzydamine Hydrochloride and Benzydamine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The solution of Benzydamine Hydrochloride in water (1 in 50) responds to the Qualitative Tests for chloride.

pH The pH of a solution obtained by dissolving 1 g of Benzydamine Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—When 1 g of Benzydamine Hydrochloride is dissolved in 10 mL of water, the resultant solution is clear.

(2) ***Heavy metals*** —Proceed with 1.0 g of Benzydamine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Primary amines***—Dissolve 50 mg of Benzydamine Hydrochloride in 10 mL of ethanol (95), and add 0.1 mL of hydrochloric acid and 2 mL of the solution of 4-dimethylaminobenzaldehyde in ethanol (95) (1 in 20). The yellow color obtained is not more intense than that obtained by treating 10 mL of the solution of 2-aminobenzoic acid in ethanol (95) (0.5 μg/mL) in the same manner.

(4) ***Related substances***—Dissolve 25 mg of Benzydamine Hydrochloride in a mixture of methanol and water (1 : 1) to make exactly 10 mL, and use this solution as the test solution. Dissolve 5 mg of Benzydamine Hydrochloride Related Substance I RS (3-dimethylaminopropyl 2-benzylaminobenzoate hydrochloride) and 12.5 mg of Benzydamine Hydrochloride Related Substance II RS (3-(1,5-dibenzyl-1*H*-indazol-3-yl)oxypropyldimethylamine hydrochloride) in a mixture of methanol and water (1 : 1) to make exactly 100 mL. Dilute 1.0 mL of this solution with a mixture of methanol and water (1 : 1) to 10 mL, and use this solution as the standard solution (1). Dissolve 2.5 mg of Benzydamine Hydrochloride Related Substance III RS (1-benzyl-1*H*-indazol-3-ol) in a mixture of methanol and water (1 : 1) to make exactly 100 mL. Pipet 1 mL of this solution, dilute with a mixture of methanol and water (1 : 1) to 10 mL, and use this solution as the standard solution (2). Dilute 0.1 mL of the test solution with a mixture of methanol and water (1 : 1) to 100 mL, and use the solution as the standard solution (3). The standard solution (4) contains equal volumes of the test solution, the standard solution (1) and the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions. Determine the area of each peak by the automatic integration method. The areas of any peak corresponding to the related substance I or the related substance II are not larger than the area of the corresponding peak from the standard solution (1) (not more than 0.2 % of the related substance I; not more than 0.5 % of the related substance II); the area of any peak corresponding to the related substance III is not larger than the area of the peak from the standard solution (2) (0.1 %); the area of any other secondary peak is not larger than the area of the peak from the standard solution (3) (0.1 %); and the sum of the areas of any such peaks is not larger than 1 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A a constant temperature of about 25 ºC.

Mobile phase: Variable mixtures of mobile A and mobile phase B, and program the chromatograph as follows.

Mobile phase A: An aqueous solution containing 0.01 mol/L potassium dihydrogen phosphate and 0.005 mol/L sodium octyl sulphate, adjusted to pH 3.0 ± 0.1 with phosphoric acid.

Mobile phase B: Methanol.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	50	50
0-20	50→30	50→70
20-22	30→50	70→50
22-30	50	50

Flow rate: 1.5 mL/min.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (4) under the above operating conditions, the retention time of benzydamine is about 10 minutes, with the resolution between the peak of benzydamine and any adjacent peak being not less than 2.5.

Loss on Drying Not more than 0.5 % (1 g, 0.67 kPa, 105 ºC, 3 hours).

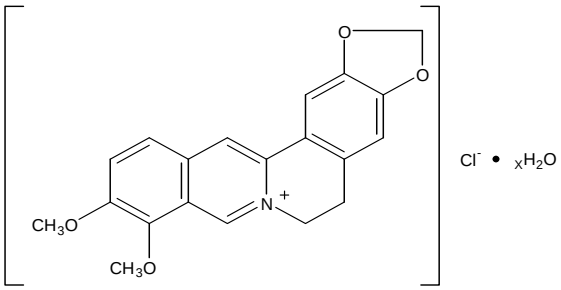
Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve 0.3 g of Benzydamine Hydrochloride, accurately weighed, in 100 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.59 mg of $C_{19}H_{23}N_3O \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Berberine Chloride Hydrate



Berberine Hydrochloride
Berberine Chloride $C_{20}H_{18}ClNO_4 \cdot \chi H_2O$

9,10-Dimethoxy-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium chloride hydrate [*141433-60-5*]

Berberine Chloride Hydrate contains not less than 95.0 % and not more than 102.0 % of berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81) calculated on the anhydrous basis.

Description Berberine Chloride Hydrate appears as

yellow crystals or crystalline powder, is odorless or has a faint, characteristic odor and has a very bitter taste. Berberine Chloride Hydrate is sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectra of the solutions of Berberine Chloride Hydrate and Berberine Chloride Hydrate RS (1 in 100000) as directed under the Ultraviolet-visible Spetrophotometry: both spectra exhibit similar intensities at the same wavelengths.

(2) Determine the infrared spectra of Berberine Chloride Hydrate and Berberine Chloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.1 g of Berberine Chloride Hydrate in 20 mL of water by warming, add 0.5 mL of nitric acid, cool and filter after allowing to stand for 10 minutes. To 3 mL of the filtrate, add 1 mL of silver nitrate TS and filter and collect the produced precipitate: the precipitate does not dissolve in dilute nitric acid, but it dissolves in an excess amount of ammonia TS.

Purity (1) ***Acid***—Shake thoroughly 0.10 g of Berberine Chloride Hydrate with 30 mL of water and filter. To the filtrate, add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the yellow color changes to an orange to red color.

(2) ***Sulfate***—Shake 1.0 g of Berberine Chloride Hydrate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL and perform the test with this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Berberine Chloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(4) ***Related substances***—Dissolve 10 mg of Berberine Chloride Hydrate in 100 mL of mobile phase and use this solution as the test solution. Pipet 4 mL of the test solution, add the mobile phase to make exactly 100 mL, use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: total of the peak areas of peaks other than berberine of the test solution is not larger than the peak area of berberine of the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of berberine obtained from 10 μL of the standard solution is about 10 % of the full scale.

Time span of measurement: About 2 times as long as the retention time of berberine, after the solvent peak.

Water 8.0 % to 12.0 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 10 mg of Berberine Chloride Hydrate and dissolve in mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Berberine Chloride Hydrate RS (separately determined water content) and dissolve in the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S of berberine in each solution.

Amount (mg) of berberine chloride ($C_{20}H_{18}ClNO_4$)
= Amount (mg) of Berberine Chloride Hydrate RS, calculated on the anhydrous basis $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 345nm).

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sufate in 1000 mL of a mixture of water and acetonitrile (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

System suitability

System performance: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, are eluted in this order with the resolution between these two peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Berberine Tannate

Berberine Tannate is a compound of berberine and tannic acid. Berberine Tannate contains not less than 27.0 % and not more than 33.0 % of berberine ($C_{20}H_{19}NO_5$: 353.37), calculated on the anhydrous basis.

Description Berberine Tannate is a yellow to pale yellow-brown powder, is odorless or has a faint, characteristic odor and is tasteless.
Berberine Tannate is practically insoluble in water, in methanol, in ethanol (95) or in ether.

Identification (1) To 0.1 g of Berberine Tannate, add 10 mL of ethanol (95) and heat in a water-bath for 3 minutes with shaking. Cool, filter and to 5 mL of the filtrate, add 1 drop of iron (III) chloride TS: a blue-green color is produced and on allowing to stand, a bluish black precipitate is formed.

(2) Dissolve 10 mg of Berberine Tannate and Berberine Tannate RS in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS and add water to make 200 mL. To each 8 mL of these solutions add water to make 25 mL. Determine the absorption spectra of the solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Berberine Tannate and Berberine Tannate RS, previously dried, as directly in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Acid***— To 0.10 g of Berberine Tannate add 30 mL of water and filter after shaking well. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the color of the solution changes from yellow to orange to red.

(2) ***Chloride***— Shake 1.0 g of Berberine Tannate with 38 mL of water and 12 mL of dilute nitric acid for 5 minutes and filter. Discard the first 5 mL of the filtrate, to 25 mL of the subsequent filtrate, add water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS by adding 6 mL of dilute nitric acid, 10 to 15 drops of bromophenol blue TS and water to make 50 mL (not more than 0.035 %).

(3) ***Sulfate***—Shake 1.0 g of Berberine Tannate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromphenol blue TS and water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Berberine Tannate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(5) ***Related substances***—Dissolve 10 mg of Berberine Tannate in 100 mL of the mobile phase and use this solution as the test solution. Pipet 4 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak areas other than berberine of the test solution is not larger than the peak area of berberine of the standard solution.

Operating conditions.

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of berberine obtained with 10 μL of this solution is equivalent to 7 to 13 % of that with 10 μL of the standard solution

Detection sensitivity: Adjust the detection sensitivity so that the peak height of berberine obtained from 10 μL of the standard solution is about 10 % of the full scale.

Time span of measurement: About 2 times as long as the retention time of berberine, after the solvent peak.

Water Not more than 6.0 % (0.7 g, volumetric titration, direct titration).

Residue on Ignition Not more than 1.0 % (1 g).

Assay Weigh accurately 30 mg of Berberine Tannate and dissolve in mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determined water content) and dissolve in the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine chloride ($C_{20}H_{19}NO_5$)
= Amount (mg) of Berberine Chloride RS,

calculated on the anhydrous basis $\times \frac{A_T}{A_S} \times 0.9504$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sufate in 1000 mL of a mixture of water and acetonitrile (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

System suitability

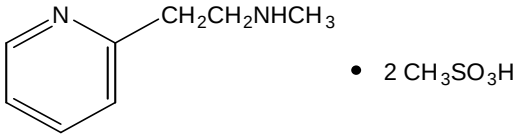
System performance: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, are eluted in this order with the resolution between these two peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Betahistine Mesilate



$C_8H_{12}N_2 \cdot 2CH_4O_3S$: 328.41

Methanesulfonic acid; *N*-methyl-2-pyridin-2-ylethanamine [*5638-76-6*]

Betahistine Mesilate, when dried, contains not less than 98.0 % and not more than 101.0 % of betahistine mesilate ($C_8H_{12}N_2 \cdot 2CH_4O_3S$).

Description Betahistine Mesilate appears as white crystals or crystalline powder.

Betahistine Mesilate is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

Betahistine Mesilate dissolves in dilute hydrochloric acid.

Betahistine Mesilate is hygroscopic.

Identification (1) Determine the absorption spectra of the solutions of Betahistine Mesilate and Betahistine Mesilate RS in 0.1 mol/L hydrochloric acid (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Betahistine Mesilate and Betahistine Mesilate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Betahistine Mesilate responds to the Qualitative Tests (2) for mesilate.

Melting Point 110 ~ 114 ºC (after drying).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Betahistine Mesilate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 50 mg of Betahistine Mesilate in 10 mL of a mixture of water and acetonitrile (63:37), and use this solution as the test solution. Pipet 1 mL of the test solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solutions as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than betahistine with the test solution is not larger than 1/10 times the peak area of betahistine with the standard solution, and the total area of the peaks other than the peak area betahistine with the test solution is not larger than 1/2 times the peak area of betahistine with the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 261 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 ºC.

Mobile phase: To 5 mL of diethyl amine and 20 mL of acetic acid (100), add water to make 1000 mL. Dissolve 2.3 g of sodium lauryl sulfate in 630 mL of this solution, and add 370 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of betahistine is about 5 minutes.

System suitability

Test for required detectability: To exactly 5 mL of the standard solution, add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 μL of

this solution is equivalent to 7 to 13 % of that with 20 μL of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution, add the mixture of water and acetonitrile (63:37) to make 50 L. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0 %.

Time span of measurement: About 3 times as long as the retention time of betohistine after the solvent peak.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, P_2O_5, 70 ºC, 24 hours).

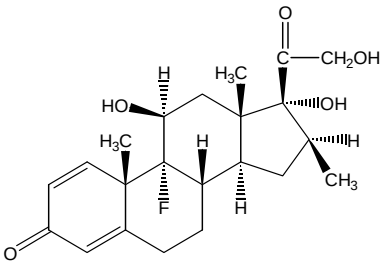
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.2 g of Betahistine Mesilate, previously dried, dissolve in 1 mL of acetic acid (100), add 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.420 mg of $C_8H_{12}N_2 \cdot 2CH_4O_3S$

Containers and Storage ***Containers***—Tight containers.

Betamethasone



$C_{22}H_{29}FO_5$: 392.46

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one [*378-44-9*]

Betamethasone, when dried, contains not less than 96.0 % and not more than 103.0 % of betamethasone ($C_{22}H_{29}FO_5$).

Description Betamethasone is a white to pale yellowish white, crystalline powder and is odorless.
Betamethasone is sparingly soluble in methanol, in ethanol (95), in acetone or in 1,4-dioxane, very slightly soluble in chloroform or in ether and practically insoluble in water.

Melting point— About 240 ºC (with decomposition).

Identification (1) Proceed 10 mg of Betamethasone as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid and prepare the test solution: the test solution so obtained responds to the Qualitative Tests for fluoride.

(2) Dissolve 1.0 mg each of Betamethasone and Betamethasone RS in 10 mL each of ethanol (95). To 2.0 mL each of these solutions, add 10 mL each of phenylhydrazine hydrochloride TS, mix by shaking, heat in a water-bath at 60 ºC for 20 minutes. After cooling, determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry using a solution, prepared with 2.0 mL of ethanol (95) in the same manner, as the blank: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Betamethasone and Betamethasone RS, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve each of Betamethasone and Betamethasone RS in acetone, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +118 ~ +126° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Betamethasone according to Method 2 and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 10 mg of Betamethasone in 5 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ether, methanol and water (385 : 75 : 40 : 6) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more in-

tense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.5 % (0.1 g, platinum crucible).

Assay Dissolve about 20 mg each of Betamethasone and Betamethasone RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = \text{Amount (mg) of Betamethasone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1750).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

System suitability

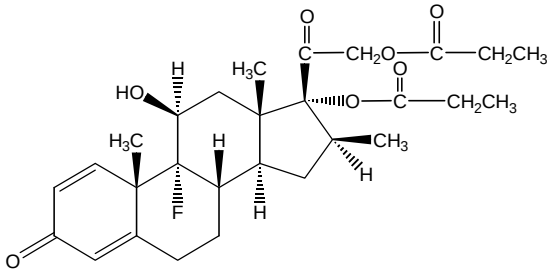
System performance: When the procedure is run with 10 μL each of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Betamethasone Dipropionate



$C_{28}H_{37}FO_7$: 504.59

[2-[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-Fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-17-propanoyloxy-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] propanoate [*5593-20-4*]

Betamethasone Dipropionate, when dried, contains not less than 97.0 % and not more than 103.0 % of betamethasone dipropionate ($C_{28}H_{37}FO_7$) and not less than 3.4 % and not more than 4.1 % of fluorine (F: 19.00).

Description Betamethasone Dipropionate is a white to pale yellowish white, crystalline powder and is odorless.

Betamethasone Dipropionate is freely soluble in acetone, in 1,4-dioxane or in chloroform, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in ether and practically insoluble in water or in hexane.

Betamethasone Dipropionate is gradually affected by light.

Identification (1) To 1 mL of a solution of Betamethasone Dipropionate in methanol (1 in 10000), add 4 mL of isoniazid TS and heat in a water-bath for 2 minutes: a yellow color is observed.

(2) Proceed with 10 mg of Betamethasone Dipropionate as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests for fluoride.

(3) Determine the absorption spectra of the solutions of Betamethasone Dipropionate and Betamethasone Dipropionate RS in methanol (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Betamethasone Dipropionate and Betamethasone Dipropionate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +63 ~ +70° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Melting Point 176 ~ 180 ºC.

Purity (1) ***Fluoride***—To 0.10 g of Betamethasone Dipropionate, add 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), shake for 10 minutes and filter through a membrane filter (0.4 μm pore size). Place 5.0 mL of the filtrate in a volumetric flask and add 10 mL of a mixture of alizalin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1), add water to make 20 mL, allow to stand for 1 hour and use this solution as the test solution. Separately, place 1.0 mL of standard fluorine solution in a volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), then 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1), proceed in the same manner as the preparation of the test solution and use this solution as the standard solution. Place 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a volumetric flask, proceed in the same manner as the preparation of the test solution and use this solution as the blank. Determine the absorbances of the test solution and the standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution is not greater than that of the standard solution (not more than 0.012 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Betamethasone Dipropionate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform the test without exposure to daylight using light-resistant vessels. Dissolve 10 mg of Betamethasone Dipropionate in 10 mL of chloroform and use this solution as the test solution. Pipet 3 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography with the test solution and the standard solution. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (7 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.2 % (0.5 g, platinum crucible).

Assay (1) ***Betamethasone Dipropionate***—Weigh accurately 15 mg of Betamethasone Dipropionate, previously dried and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution and dilute with methanol to make exactly 50 mL. Determine the absorbance A of this solution at the wavelength of a maximum absorption at about 239 nm as directed under Ultraviolet-visible Spectrophotometry.

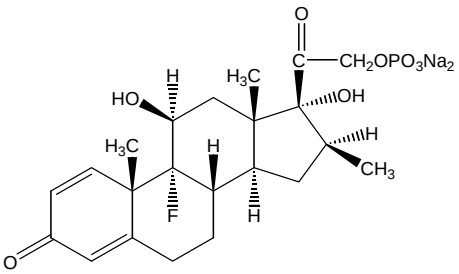
$$\text{Amount (mg) of betamethasone dipropionate } (C_{28}H_{37}FO_7) = \frac{A}{312} \times 10000$$

(2) ***Fluorine***—Weigh accurately 10 mg of Betamethasone Dipropionate, previously dried and proceed as directed in the determination for fluorine under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Betamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$: 516.41

Disodium [2-[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a] phenanthren-17-yl]-2-oxoethyl] phosphate [*151-73-5*]

Betamethasone Sodium Phosphate contains not less than 97.0 % and not more than 103.0 % of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), calculated on the anhydrous basis.

Description Betamethasone Sodium Phosphate is a white to pale yellowish white, crystalline powder or mass and is odorless.

Betamethasone Sodium Phosphate is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in ether.

Betamethasone Sodium Phosphate is hygroscopic.

Melting point —About 213 ºC (with decomposition).

Identification (1) Dissolve 2 mg of Betamethasone Sodium Phosphate in 2 mL of sulfuric acid: a brown color develops and gradually changes to blackish

brown.

(2) Prepare the test solution with 10 mg of Betamethasone Sodium Phosphate as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests (2) for fluoride.

(3) Take 40 mg of Betamethasone Sodium Phosphate in a platinum crucible and carbonize by heating. After cooling, add 5 drops of nitric acid and incinerate by heating. To the residue, add 10 mL of diluted nitric acid (1 in 50) and boil for several minutes. After cooling, the solution responds to the Qualitative Tests (2) for phosphate. Neutralize the test solution with ammonia TS: the solution responds to the Qualitative Tests for sodium salt, and to the Qualitative Tests (1) or (3) for phosphate.

(4) Determine the infrared spectra of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +99 ~ +105° (0.1 g, calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH Dissolve 0.10 g of Betamethasone Sodium Phosphate in 20 mL of water: the pH of this solution is between 7.5 and 9.0.

Purify (1) ***Clarity and color of solution***—Dissolve 0.25 g of Betamethasone Sodium Phosphate in 10 mL of water: the solution is clear and colorless.

(2) ***Free phosphoric acid***—Weigh accurately about 20 mg of Betamethasone Sodium Phosphate, dissolve in 20 mL of water and use this solution as the test solution. Separately, pipet 4 mL of phosphoric acid standard solution, add 20 mL of water and use this solution as the standard solution,. To each of the test solution and the standard solution, add exactly 7 mL of dilute sulfuric acid, exactly 2 mL of ammonium molybdate-sulfuric acid TS and exactly 2 mL of *p*-methylaminophenol sulfate TS, shake well and allow to stand at 20 ± 1 °C for 15 minutes. To each, add water to make exactly 50 mL and allow to stand at 20 ± 1 °C for 15 minutes. Perform the test with the test solution and the standard solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 20 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 730 nm: the amount of free phosphoric acid is not more than 0.5 %.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 10.32$$

W: Amount (mg) of Betamethasone Sodium Phosphate, calculated on the anhydrous basis.

(3) ***Betamethasone***—Dissolve 20 mg of Betamethasone Sodium Phosphate in exactly 2 mL of methanol and use this solution as the test solution. Separately, dissolve 20 mg of Betamethasone RS in exactly 10 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a freshly prepared mixture of 1-butanol, water and acetic anhydride (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Water Not more than 10.0 % (0.2 g, volumetric titration, back titration).

Assay Weigh accurately about 20 mg each of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate RS (determined its water content before using in the same manner as Betamethasone Sodium Phosphate) and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone phosphate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of betamethasone sodium phosphate } (C_{22}H_{28}FNa_2O_8P) = \text{Amount (mg) of dried Betamethasone Sodium Phosphate RS} \times \frac{Q_T}{Q_S}$$

Internal Standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC .

Mobile phase: Dissolve 1.6 g of tetra-*n*-butyl-

ammonium bromide, 3.2 g of dibasic sodium phosphate and 6.9 g of monobasic potassium phosphate in 1000 mL of water and add 1500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of betamethasone phosphate is about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the procedure is run 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deivation o the ratios of the peak area of betamethasone phosphate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Betamethasone Tablets

Betamethasone Tablets contain not less than 90.0 % and not more than 107.0 % of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$: 392.46).

Method of Preparation Prepare as directed under Tablets, with Betamethasone.

Identification Pulverize Betamethasone Tablets. To a portion of the powder, equivalent to 2 mg of Betamethasone according to the labeled amount, add 20 mL of methanol, shake for 5 minutes, and filter. Evaporate the filtrate on a water bath to dryness, dissolve the residue after cooling in 2 mL of methanol, filter if necessary, and use the solution as the test solution. Separately, dissolve 2 mg of Betamethasone RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on the plate of silica gel with a fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots from the test solution and the standard solution show the same R_f value.

Dissolution Test Perform the test with 1 tablet of Betamethasone Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using the mixture of 900 mL of water as the dissolution solution. Withdraw 20 mL or more of the dissolution solution 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of betamethasone ($C_{22}H_{29}FO_5$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of betamethasone for the test solution and the standard solution. The dissolution rate in 30 minutes is not less than 85 %.

Dissolution rate (%) with respect to the labeled amount of betamethasone ($C_{22}H_{29}FO_5$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{9}{5}$$

W_S: Amount (mg) of Betamethasone RS

C: Labeled amount (mg) of betamethasone ($C_{22}H_{29}FO_5$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 7 minutes.

System suitability

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 30 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone is not more than 1.0 %.

Uniformity of Dosage Units It meets the requirement, when the content uniformity test is performed according to the following method.

To 1 tablet of Betamethasone Tablets, add V mL of water so that each mL contains about 50 μg of betamethasone ($C_{22}H_{29}FO_5$) according to the labeled amount, add exactly an amount of the internal standard solution equivalent to 2 mL per 50 μg of betamethasone in this

solution, shake vigorously for 10 minutes, centrifuge, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of water and 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 50 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard for the test solution and the standard solution.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = \text{Amount (mg) of Betamethasone RS} \times \frac{Q_T}{Q_S} \times \frac{V}{400}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 40000)

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.

System suitability

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is run 6 times with 50 µL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0 %.

Assay Weigh accurately not less than 20 Betamethasone Tablets and powder. Weigh accurately a portion of powder, equivalent to about 5 mg of betamethasone($C_{22}H_{29}FO_5$), add 25 mL of water, then add exactly 50 mL of the internal standard solution, and shake vigorously for 10 minutes. Filter through a membrane filter with a pore size of not more than 0.5 µm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of water and 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard for the test solution and the standard solution.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = \text{Amount (mg) of Betamethasone RS} \times \frac{Q_T}{Q_S} \times \frac{1}{4}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

System suitability

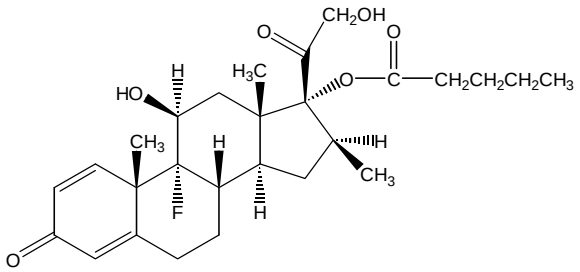
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, Betamethasone and internal standard is eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Betamethasone Valerate



$C_{27}H_{37}FO_6$: 476.58

[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-Fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] pentanoate [*2152-44-5*]

Betamethasone Valerate, when dried, contains not less than 97.0 % and not more than 103.0 % of betamethasone valerate ($C_{27}H_{37}FO_6$).

Description Betamethasone Valerate is a white crystalline powder and is odorless.
Betamethasone Valerate is freely soluble in chloroform, soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in ether and practically insoluble in water.

Melting point— About 190 ºC (with decomposition).

Identification (1) Proceed with 10 mg of Betamethasone Valerate as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid and prepare the test solution: the test solution responds to the Qualitative Tests for fluoride.

(2) Determine the infrared spectra of Betamethasone Valerate and Betamethasone Valerate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +77 ~ +83° (after drying, 0.10 g, methanol, 20 mL, 100 mm)

Purity ***Related substances***—Perform this procedure without exposure to light. Dissolve 20 mg of Betamethasone Valerate in 5 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.2 % (0.5 g, platinum crucible).

Assay Dissolve about 10 mg each of Betamethasone Valerate and Betamethasone Valerate RS, previously dried and accurately weighed, in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL each of the internal standard solution and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operation conditions and calculate the ratios, Q_T and Q_S, of the peak area of Betamethasone Valerate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of Betamethasone Valerate } (C_{27}H_{37}FO_6) = \text{Amount (mg) of Betamethasone Valerate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isoamyl benzoate in methanol (1 in 1000).

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 20 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and water (7 : 3).

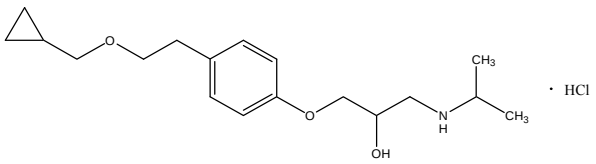
Flow rate: Adjust the flow rate so that the retention time of Betamethasone Valerate is about 10 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions and calculate the resolution. Use a column giving elution of Betamethasone Valerate and the internal standard in this order with the resolution between their peaks being not less than 5.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Betaxolol Hydrochloride



$C_{18}H_{29}NO_3 \cdot HCl$: 343.89

1-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-(propan-2-ylamino)propan-2-ol hydrochloride
[*63659-19-8*]

Betaxolol Hydrochloride contains not less than 99.0 % and not more than 101.0 % of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$), calculated on the dried basis.

Description Betaxolol Hydrochloride is a white, crystalline powder.
Betaxolol Hydrochloride is very soluble in water, and freely soluble in methanol, in ethanol (95), or in acetic acid (100).

A solution of Betaxolol Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Betaxolol Hydrochloride and Betaxolol Hydrochloride RS in ethanol (95) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Betaxolol Hydrochloride and Betaxolol Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Betaxolol Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting Point 114 ~ 117 °C

pH The pH of a solution obtained by dissolving 1 g of Betaxolol Hydrochloride in 50 mL of water is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Betaxolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Betaxolol Hydrochloride according to Method 4 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Betaxolol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) ***Related substance I***—Dissolve 0.10 g of Betaxolol Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Pipet 3 mL of this solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water, and acetic acid (100) (10 : 3 : 3) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 1 hour: the number of spots other than the principal spot obtained from the test solution is not more than 3, and they are not more intense than the spot from the standard solution.

(5) ***Related substance II***—Dissolve 0.10 g of Betaxolol Hydrochloride in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than betaxolol obtained from the test solution is not larger than the peak area of betaxolol from the standard solution, and the total area of the peaks other than the peak of betaxolol from the test solution is not larger than 2 times the peak area of betaxolol from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of diluted 0.05 mol/L potassium dihydrogen phosphate TS (1 in 2) with the pH adjusted to 3.0 with 1 mol/L hydrochloric acid TS, acetonitrile, and methanol (26 : 7 : 7)

Flow rate: Adjust the flow rate so that the retention time of betaxolol is about 9 minutes.

System suitability

Test for required detectability: Pipet 4 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of betaxolol obtained from 10 μL of this solution is equivalent to 14 to 26 % of that from 10 μL of the standard solution.

System performance: Dissolve 50 mg of Betaxolol Hydrochloride and 5 mg of 2-naphthol in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, betaxolol and 2-naphthol are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betaxolol is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of betaxolol beginning after the solvent peak

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours)

Residue on Ignition Not more than 0.1 % (1 g)

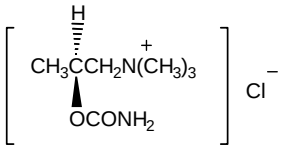
Assay Dissolve about 0.3 g of Betaxolol Hydrochloride, accurately weighed, in 50 mL of acetic acid (100), add 7 mL of mercuric acetate TS, and titrate with 0.1mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 34.39 mg of $C_{18}H_{29}NO_3 \cdot HCl$.

Containers and Storage ***Containers***—Tight containers.

Bethanechol Chloride



and enantiomer

$C_7H_{17}ClN_2O_2$: 196.68

2-Carbamoyloxypropyl(trimethyl)azanium chloride [*590-63-6*]

Bethanechol Chloride, when dried, contains not less than 98.0 % and not more than 101.0 % of bethanechol chloride ($C_7H_{17}ClN_2O_2$).

Description Bethanechol Chloride appears as colorless or white crystals or a white crystalline powder.
Bethanechol Chloride is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).
Bethanechol Chloride is hygroscopic.
A solution of Bethanechol Chloride (1 in 10) shows no optical rotation.

Identification (1) To 2 mL of a solution of Bethanechol Chloride (1 in 40), add 0.1 mL of a solution of cobalt (II) chloride hexahydrate (1 in 100), then add 0.1 mL of potassium hexacyanoferrate (II) TS: a green color is observed, and almost entirely fades within 10 minutes.

(2) To 1 mL of a solution of Bethanechol Chloride (1 in 100), add 0.1 mL of iodine TS: a brown precipitate is produced, and the solution shows a greenish brown color.

(3) Determine the infrared spectra of Bethanechol Chloride and Bethanechol Chloride RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Bethanechol Chloride (1 in 100) responds to the Qualitative Tests for chloride.

Melting Point 217 ºC ~ 221 ºC (after drying)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Bethanechol Chloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 1.0 g of Bethanechol Chloride in 2.5 mL of water, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium chloride acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105 ºC for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate, and allow to stand for 30 minutes: the spot other than the principal spot from the test solution is not more than intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Chloride Content Weigh accurately 0.4 g of Bethanechol Chloride, previously dried, and dissolve in 30 mL of water. Add 40.0 mL of 0.1 mol/L silver nitrate TS, then add 3 mL of nitric acid and 5 mL of nitrobenzene, and shake for a few minutes. Add 2 mL of ammonium iron (III) sulfate TS and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS. The content of chloride is between 17.7 and 18.3 %.

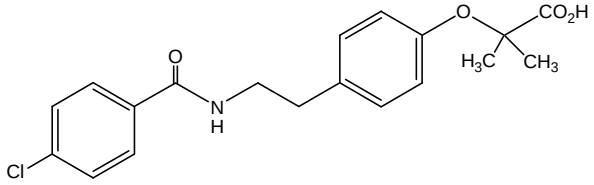
Each mL of 0.1 mol/L silver nitrate TS
= 3.545 mg of Cl

Assay Weigh accurately 0.4 g of Bethanechol Chloride, previously dried, dissolve in 2 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.668 mg of $C_7H_{17}ClN_2O_2$

Containers and Storage ***Containers***—Tight containers.

Bezafibrate



$C_{19}H_{20}ClNO_4$: 361.82

2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid [*41859-67-0*]

Bezafibrate contains not less than 98.5 % and not more than 101.0 % of bezafibrate ($C_{19}H_{20}ClNO_4$), calculated on the dried basis.

Description Bezafibrate is a white, crystalline powder.
Bezafibrate is freely soluble in *N,N*-dimethylformamide, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Bezafibrate and Bezafibrate RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bezafibrate and Bezafibrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Bezafibrate as directed under the Flame Coloration Test (2): it shows a green color.

Melting Point 181 ~ 186 ºC.

Purity (1) ***Chlorides***—Dissolve 3.0 g of Bezafibrate in 15 mL of *N,N*-dimethylformamide, dilute with water to 60 mL, shake well, allow to stand for at least 12 hours, and filter. To 40 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Separately, mix 0.70 mL of 0.01 mol/L hydrochloric acid, 10 mL of *N,N*-dimethyl-formamide, and 6 mL of dilute nitric acid. Dilute this solution with water to 50 mL, and use this solution as the control solution (not more than 0.012 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Bezafibrate, according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Bezafibrate in 35 mL of methanol, add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 50 mL, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add 70 mL of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks having the relative retention times of about 0.65 and 1.86 with respect to bezafibrate obtained from the test solution are not larger than 0.5 times the peak area of bezafibrate from the standard solution; the area of the peak other than those and other than bezafibrate from the test solution is not larger than 0.2 times the peak area of bezafibrate from the standard solution; and the sum of the total area of the peaks other than the peak of bezafibrate from the test solution is not larger than 0.75 times the peak area of bezafibrate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust the flow rate so that the retention time of bezafibrate is about 6 minutes.

System suitability

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) (7 : 3) to make exactly 50 mL. Confirm that the peak area of bezafibrate obtained with 5 μL of this solution is equivalent to 7 to 13 % of that with 5 μL of the standard solution.

System performance: Dissolve 20 mg of Bezafibrate and 10 mg of 4-chlorobenzoate in 70 mL of methanol, and add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make exactly 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, 4-chlorobenzoate and bezafibrate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bezafibrate is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of bezafibrate beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

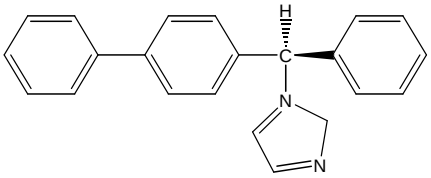
Residue on Ignition Not more than 0.1 % (1 g).

Assay Accurately weigh about 0.7 g of Bezafibrate, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.183 mg of $C_{19}H_{20}ClNO_4$

Containers and Storage ***Containers***—Tight containers.

Bifonazole



and enantiomer

$C_{22}H_{18}N_2$: 310.39

1-[[(1,1'-Biphenyl)-4-yl]phenylmethyl]imidazole
[*60628-96-8*]

Bifonazole, when dried, contains not less than 98.5 % and not more than 101.0 % of bifonazole ($C_{22}H_{18}N_2$).

Description Bifonazole is a white to pale yellow powder, is odorless and tasteless.
Bifonazole is freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.
A solution of Bifonazole in methanol (1 in 100) does not show optical rotation.

Identification (1) Determine the absorption spectra of the solutions of Bifonazole and Bifonazole RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bifonazole and Bifonazole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 147 ~ 151 ºC

Purity (1) ***Chloride***—To 2.0 g of Bifonazole add 40 mL of water, warm for 5 minutes, cool and filter. To 10 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(2) ***Sulfate***—To 10 mL of the filtrate obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Bifonazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Related substances***—Perform this test without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bifonazole in 10 mL of methanol and use this solution as the test solution. Pipet 3 mL of the test solution and add methanol to make exactly 100 mL. Pipet 25 mL and 5 mL of this solution, add methanol to make exactly 50 mL each and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (49 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot with R_f value of about 0.20 from the test solution is not more intense than the spot from the standard solution (1). And the spots other than the spot mentioned above and the principal spot from the test solution are not more intense than the spot from the standard solution (2).

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

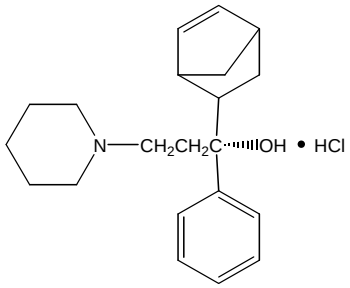
Assay Weigh accurately 0.15 g of Bifonazole, previously dried and dissolve in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution in a glass-stoppered Erlenmeyer flask, add 10 mL of water, 5 mL of dilute sulfuric acid and 25 mL of dichloromethane and add 2 to 3 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator and titrate, while shaking vigorously, with 0.01 mol/L sodium lauryl sulfate VS by a buret with 0.02-mL minimum graduation. The end point is reached when the color of the dichloromethane layer changes from yellow to orange after dropwise addition of 0.01 mol/L sodium lauryl sulfate VS, strong shaking and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS
= 3.1039 mg of $C_{22}H_{18}N_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Biperidene Hydrochloride



and enantiomer

$C_{21}H_{29}NO \cdot HCl$: 347.92

1-(Bicyclo[2.2.1]hept-2-en-5-yl)-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride [*1235-82-1*]

Biperiden Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of biperiden hydrochloride ($C_{21}H_{29}NO \cdot HCl$).

Description Biperiden Hydrochloride is a white to brownish and yellowish white, crystalline powder.
Biperiden Hydrochloride is freely soluble in formic acid, slightly soluble in methanol or in ethanol (95) and practically insoluble in ether..

Melting point—About 270 ºC (with decomposition).

Identification (1) Dissolve 20 mg of Biperiden Hydrochloride in 5 mL of phosphoric acid: a green color is observed.

(2) Dissolve 10 mg of Biperiden Hydrochloride in 5 mL of water by heating, cool and add 5 to 6 drops of bromine TS: a yellow precipitate is produced.

(3) Determine the absorption spectra of the solutions of Biperiden Hydrochloride and Biperiden Hydrochloride RS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Biperiden Hydrochloride and Biperiden Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(5) Dissolve 20 mg of Biperiden Hydrochloride in 10 mL of water by heating and cool: the solution responds to the Qualitative Tests for chloride.

Purity (1) ***Acid or alkali***—To 1.0 g of Biperiden Hydrochloride, add 50 mL of water, shake vigorously, filter and to 20 mL of the filtrate, add 1 drop of methyl red TS: no red or yellow color is observed.

(2) ***Heavy metals***—Proceed with 1.0 g of Biperiden Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Biperiden Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Biperiden Hydrochloride in 20 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 50 µL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (80 : 15 : 2) to a distance of about 15 cm and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

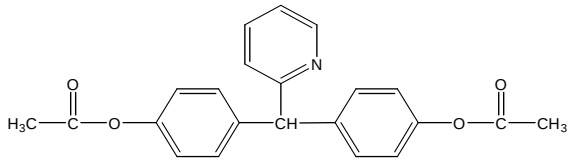
Assay Weigh accurately 0.4 g of Biperiden Hydrochloride, previously dried and dissolve in 5 mL of formic acid, add 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometic titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.792 mg of $C_{21}H_{29}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Bisacodyl



$C_{22}H_{19}NO_4$: 361.39

4,4'-(2-Pyridinylmethylene)bisphenol-1,1'-diacetate [*603-50-9*]

Bisacodyl, when dried, contains not less than 98.5 % and not more than 101.0 % of bisacodyl ($C_{22}H_{19}NO_4$).

Description Bisacodyl is a white, crystalline powder.
Bisacodyl is freely soluble in acetic acid (100), soluble in acetone, slightly soluble in ethanol (95) or in ether and practically insoluble in water.
Bisacodyl dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectra of the solutions of Bisacodyl and Bisacodyl RS in ethanol (95) (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bisacodyl and Bisacodyl RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 132 ~ 136 ºC

Purity (1) ***Chloride***—Dissolve 1.0 g of Bisacodyl in 30 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.01 mol/L hydrochloric acid VS, add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012 %).

(2) ***Sulfate***—Dissolve 1.0 g of Bisacodyl in 2 mL of dilute hydrochloric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.005 mol/L sulfuric acid VS, add 2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.017 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Bisacodyl according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Bisacodyl in 10 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, Develop the plate with a mixture of 2-butanone, chloroform and xylene (1 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.5 g of Bisacodyl, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-yellow to green (indicator: 0.5 mL of 1-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.139 mg of $C_{22}H_{19}NO_4$

Containers and Storage ***Containers***—Well-closed containers.

Bisacodyl Suppositories

Bisacodyl Suppositories contain not less than 90.0 % and not more than 110.0 % of the labeled amount of bisacodyl ($C_{22}H_{19}NO_4$: 361.39).

Method of Preparation Prepare as directed under Suppositories, with Bisacodyl.

Identification (1) To a portion of Bisacodyl Suppositories, equivalent to 6 mg of Bisacodyl according to the labeled amount, add 20 mL of ethanol (95), warm in a water-bath for 10 minutes, shake vigorously for 10 minutes and allow to stand in ice-water for 1 hour. Collect the clear supernatant liquid after centrifugation, filter the supernatant and to 2 mL of the filtrate, add ethanol (95) to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 265 nm.

(2) Use the filtrate obtained in (1) as the test solution, Separately, dissolve 6 mg of Bisacodyl RS in 20 mL of ethanol (95) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, chloroform and xylene (1 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution and the standard solution show the same R_f value.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content Uniformity Test.

To 1 suppository of Bisacodyl Suppositories add tetrahydrofuran to make a solution containing about 0.2 mg of bisacodyl ($C_{22}H_{19}NO_4$) in each mL, warm to 40 °C, and shake to dissolve. After cooling, add tetrahydrofuran to make exactly V mL so that each mL contains about 10 μg of bisacodyl ($C_{22}H_{19}NO_4$). Pipet 5 ml of this solution, and proceed as directed in the Assay.

Amount (mg) of bisacodyl ($C_{22}H_{19}NO_4$)

$$= \text{Amount (mg) of Bisacodyl RS} \times \frac{Q_T}{Q_S} \times \frac{V}{50}$$

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100000)

Assay Weigh accurately not less than 20 Bisacodyl Suppositories, make fine fragments carefully and mix uniformly. Weigh accurately a portion of the fragments, equivalent to about 10 mg of bisacodyl ($C_{22}H_{19}NO_4$), add 40 mL of tetrahydrofuran, warm to 40 ºC, dissolve by shaking, cool and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and add the mobile phase to make 100 mL. Cool this solution in ice for 30 minutes, centrifuge, filter the clear supernatant liquid through a membrane filter with a pore size of 0.5 μm, discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 10 mg of Bisacodyl RS, previously dried at 105 ºC for 2 hours and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, proceed in the same manner as the test solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of bisacodyl to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = \text{Amount (mg) of Bisacodyl RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of 0.01 mol/L citric acid TS, acetonitrile and methanol (2 : 1 : 1).

Flow rate: Adjust the flow rate so that the retention time of bisacodyl is about 8 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of bisacodyl to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Bisacodyl Tablets

Bisacodyl Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of bisacodyl ($C_{22}H_{19}NO_4$: 361.39).
Bisacodyl Tablets are enteric coated.

Method of Preparation Prepare as directed under Tablets, with Bisacodyl.

Identification To a quantity of powdered Bisacodyl Tablets, equivalent to about 0.3 g of Bisacodyl according to the labeled amount, add 100 mL of acetone, shake, heat in a water-bath to boiling, filter and evaporate to about 20 mL. Add 200 mL of water, evaporate acetone in a water-bath, under the nitrogen gas. Cool, filter with glass filtrator (G4) after 30 minutes and discard the filtrate. Dissolve the residue with 50 mL of acetone, evaporate to about 15 mL, add about 75 mL of water, heat in a water-bath for 15 minutes and cool. Crystallize by scratching the wall of beaker, filter, dry at 105 ºC for about 15 minutes and proceed the test with the residue as directed in the Identification (2) under Bisacodyl.

Disintegration Test When the procedure is run as directed for the enteric coated tablets, the tablets do not disintegrated after 60 minutes in the first fluid, but then disintegrated within 45 minutes in the second fluid.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately not less than 20 Bisacodyl Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of bisacodyl ($C_{22}H_{19}NO_4$), add 10 mL of water, shake thoroughly for 15 minutes and shake vigorously for 15 minutes, add 30 mL of acetonitrile, shake for 15 minutes and shake vigorously for 15 minutes, add acetonitrile to make 50 mL, mix and filter. Discard the first 10 mL of the filtrate and exactly take the subsequent 5 mL of the filtrate. Add exactly 5 mL of the internal standard. Use the this solution as the test solution. Separately, Bisacodyl RS, dried at 105 ºC for 2 hours, weigh accurately 25 mg, and add acetonitrile to make exactly 50 mL. Take exactly 5 mL of this solution, and treat as directed to prepare the test solution. Use this solution as the standard solution. Perform the test with each 20 μL of the test solution and the standard solution, as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Biscodyl to that of the internal standard for the test solution and standard solution, respectively.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = \text{Amount (mg) of Bisacodyl RS} \times \frac{Q_T}{Q_S}$$

Operating conditions

Proceed as directed in the operating conditions in the Assay under Bisacodyl Suppositories

System suitability

Syetem performance: When the procedure is run with 20 mL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Not exceeding 30 ºC.

Bismuth Subgallate

Bismuth Subgallate, when dried, contains not less than 47.0 % and not more than 51.0 % of bismuth (Bi: 208.98).

Description Bismuth Subgallate is a yellow powder, is odorless and tasteless.

Bismuth Subgallate is practically insoluble in water, in ethanol (95) or in ether.

Bismuth Subgallate dissolves in warm dilute hydrochloric acid, in dilute nitric acid or in dilute sulfuric acid. Bismuth Subgallate dissolves in sodium hydroxide TS, forming a clear, yellow solution, which turns red immediately.

Bismuth Subgallate is affected by light.

Identification (1) Ignite 0.5 g of Bismuth Subgallate: it chars at first and leaves finally a yellow residue. The residue responds to the Qualitative Tests for bismuth salt.

(2) To 0.5 g of Bismuth Subgallate, add 25 mL of water and 20 mL of hydrogen sulfide TS and shake well. Filter off the blackish brown precipitate and add 1 drop of iron (III) chloride TS to the filtrate: a blue-black color is produced.

Purity (1) ***Clarity of solution***— Dissolve 1.0 g of Bismuth Subgallate in 40 mL of diluted sodium hydroxide TS (1 in 8): the solution is clear.

(2) ***Nitrate***—To 0.5 g of Bismuth Subgallate, add 5 mL of dilute sulfuric acid and 25 mL of ferrous sulfate TS, shake well and filter. Superimpose carefully 5 mL of the filtrate on sulfuric acid: no red-brown color develops at the zone of contact.

(3) ***Sulfate***—Ignite 3.0 g of Bismuth Subgallate in a porcelain crucible and cautiously dissolve the residue in 2.5 mL of nitric acid by warming. Pour the solution into 100 mL of water, shake and filter. Evaporate 50 mL of the filtrate in a water-bath to 15 mL. Add water to make 20 mL, filter again and use the filtrate as the test solution. To 5 mL of the test solution, add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(4) ***Ammonium***—Dissolve 1.0 g of Bismuth Subgallate in 5 mL of sodium hydroxide TS and heat: the gas evolved does not change moistened red litmus paper to blue.

(5) ***Copper***—To 5 mL of the test solution obtained in (2), add 1 mL of ammonia TS and filter: no blue color develops in the filtrate.

(6) ***Lead***—Ignite 1.0 g of Bismuth Subgallate at about 500 ºC in a porcelain crucible, dissolve the residue in a smallest possible amount of nitric acid added drop-wise, evaporate over a low flame to dryness and cool. Add 5 mL of a solution of potassium hydroxide (1 in 6) to the residue, boil carefully for 2 minutes, cool and centrifuge. Take the clear supernatant liquid in a test tube, add 10 drops of potassium chromate TS and acidify the solution by adding acetic acid (100) drop-wise: neither turbidity nor a yellow precipitate is produced.

(7) ***Silver***—To 5 mL of the test solution obtained in (2), add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(8) ***Alkaline earth metals and alkali metals***—Boil 1.0 g of Bismuth Subgallate with 40 mL of diluted acetic acid (1 in 2) for 2 minutes, cool, add water to make 40 mL and filter. To 20 mL of the filtrate, add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter the precipitate produced and wash with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid and evaporate to dryness. Ignite as directed under the Residue on Ignition: the weight of the residue does not more than 5.0 mg.

(9) ***Arsenic***—Mix well 0.20 g of Bismuth Subgallate with 0.20 g of calcium hydroxide and ignite the mixture. Dissolve the residue in 5 mL of dilute hydrochloric acid, use this solution as the test solution and perform the test (not more than 10 ppm).

(10) ***Gallic acid***—To 1.0 g of Bismuth Subgallate, add 20 mL of ethanol (95), shake for 1 minute and filter. Evaporate the filtrate in a water-bath to dryness: the weight of the residue does not more than 5.0 mg.

Loss on Drying Not more than 6.0 % (1 g, 105 ºC, 3 hours).

Assay Weigh accurately 0.5 g of Bismuth Subgallate, previously dried, ignite at about 500 ºC for 30 minutes and cool. Dissolve the residue in 5 mL of diluted nitric acid (2 in 5) by warming and add water to make exactly 100 mL. Measure exactly 30 mL of this solution,

add 200 mL of water and titrate with 0.02 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 2 to 3 drops of xylenol orange TS).

Each mL of 0.02 mol/L
disodium ethylenediaminetetraacetate VS
= 4.180 mg of Bi

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Bismuth Subnitrate

Bismuth Subnitrate, when dried, contains not less than 71.5 % and not more than 74.5 % of bismuth (Bi: 208.98).

Description Bismuth Subnitrate is a white powder.
Bismuth Subnitrate is practically insoluble in water, in ethanol (95) or in ether.
Bismuth Subnitrate readily dissolves in hydrochloric acid or nitric acid without effervescence.
Bismuth Subnitrate is slightly hygroscopic and changes moistened blue litmus paper to red.

Identification Bismuth Subnitrate responds to the Qualitative Tests for bismuth salt and for nitrate.

Purity (1) ***Chloride***—Dissolve 0.7 g of Bismuth Subnitrate in 2 mL of water and 2 mL of nitric acid and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of nitric acid on a water-bath to dryness, add 0.70 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035 %).

(2) ***Sulfate***—Dissolve 3.0 g of Bismuth Subnitrate in 3.0 mL of warmed nitric acid, pour this solution into 100 mL of water and shake. Concentrate the filtrate in a water-bath to 30 mL, filter and use this filtrate as the test solution. To 5 mL of the test solution, add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) ***Ammonium***—Boil 0.10 g of Bismuth Subnitrate with 5 mL of sodium hydroxide TS: the gas evolved does not change moistened red litmus paper to blue.

(4) ***Copper***—To 5 mL of the test solution obtained in (2), add 2 mL of ammonia TS and filter: no blue color develops.

(5) ***Lead***—To 1.0 g of Bismuth Subnitrate, add 5 mL of a solution of sodium hydroxide (1 in 6), boil carefully for 2 minutes, cool and centrifuge. Transfer the clear supernatant liquid to a test tube, add 10 drops of potassium chromate TS and add drop-wise acetic acid (100) to render the solution acid: no turbidity or yellow precipitate is produced.

(6) ***Silver***—To 5 mL of the test solution obtained in (2), add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(7) ***Alkaline earth metals and alkali metals***— Boil 2.0 g of Bismuth Subnitrate with 40 mL of diluted acetic acid (1 in 2) for 2 minutes, cool, add water to make 40 mL and filter. To 20 mL of the filtrate, add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter and wash the residue with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, evaporate to dryness and ignite as directed under the Residue on Ignition: the residue does not exceed 5.0 mg.

(8) ***Arsenic***—To 0.20 g of Bismuth Subnitrate, add 2 mL of sulfuric acid, heat until white fumes evolve, dilute cautiously with water to make 5 mL, use this solution as the test solution and perform the test (not more than 10 ppm).

(9) ***Carbonate***—To 3 g of Bismuth Subnitrate add 3 mL of warm nitric acid: almost no effervescence occurs. Add this solution to 100 mL of water: a white precipitate is produced.

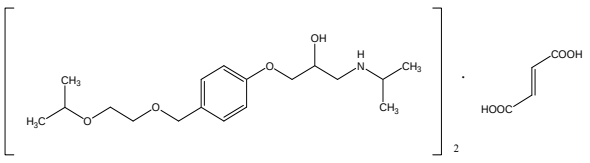
Loss on Drying Not more than 3.0 % (2 g, 105 ºC, 2 hours).

Assay Weigh accurately 0.4 g of Bismuth Subnitrate, previously dried, dissolve in 5 mL of diluted nitric acid (2 in 5) by warming and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 200 mL of water and titrate with 0.02 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 5 drops of xylenol orange TS).

Each mL of 0.02 mol/L
disodium ethylenediaminetetraacetate VS
= 4.180 mg of Bi

Containers and Storage ***Containers***—Well-closed containers.

Bisoprolol Fumarate



$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$: 766.96

2-Hydroxy-3-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-*N*-[(1-methylethyl)propanamine (2*E*)-2-butenedioate
[*104344-23-2*]

Bisoprolol Fumarate contains not less than 97.5 % and not more than 102.0 % of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$, calculated on the anhydrous basis.

Description Bisoprolol Fumarate is a white crystalline powder.
Bisoprolol Fumarate is very soluble in water or in methanol, and freely soluble in ethanol (99.5) or in acetic acid (100).
A solution of Bisoprolol Fumarate (1 in 10) shows no optical rotation.

Identification (1) Determine the infrared spectra of Bisoprolol Fumarate and Bisoprolol Fumarate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak from the test solution corresponds to that from the standard solution, as obtained in the *Assay.*

Melting Point 101 ~ 105 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: −2 ~ +0.2° (0.2 g, methanol 20 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Bisoprolol Fumarate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Fumaric acid***—Dissolve about 0.5 g of Bisoprolol Fumarate, accurately weighed, in 70 mL of ethanol (99.5). Add 8.0 mL of 0.1 mol/L tetrabutylammonium hydroxide VS, stir for 2 minutes, and titrate with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction. Not less than 14.8 % and not more than 15.4 % of fumaric acid is found, calculated on the anhydrous basis.

Each mL of 0.1 mol/L
tetrabutylammonium hydroxide VS
= 5.804 mg of fumaric acid ($C_4H_4O_4$)

(3) ***Related substances*** —Prepare the test solution as directed in the Assay. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the procedure in the Assay, and measure peak areas. When the percentage of total related substances in the portion of Bisoprolol Fumarate is calculated, not more than 0.5 % of total related substances is found.

$$\text{Amount (\%) of total related substances} = 100 \times \frac{A_i}{A_S}$$

A_i: sum of all the peak areas, excluding the peak areas of fumaric acid and bisoprolol.

A_S: sum of all the peak areas in the chromatograpm.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 80 °C, 5 hours)

Residue on Ignition **Not more than 0.1 % (1 g).**

Assay **Dissolve 50 mg** of Bisoprolol Fumarate, accurately weighed, in a mixture of acetonitrile and water (35:65) to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Bisoprolol Fumarate RS, dissolve in 35 % acetonitrile solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of Bisoprolol Fumarate.

Amount (mg) of bisoprolol fumarate

$$[(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4] = 50 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Bisoprolol Fumarate RS in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: To 1000 mL of 35 % acetonitrile solution, add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid, mix, and filter.

Flow rate: 1.0 mL/min.

System suitability

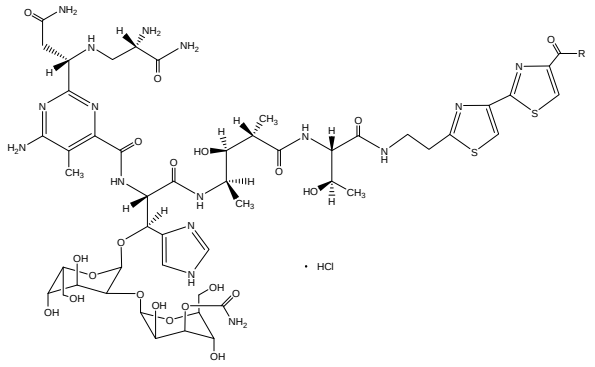
System performance: Dissolve 25.0 mg of propranolol hydrochloride and 50.0 mg of Bisoprolol Fumarate in 35 % acetonitrile solution to make 50 mL. When the procedure is run with 10 μL of this solution as directed in the Liquid chromatography under the above operating conditions, the resolution between the peaks of bisoprolol and propranolol is not less than 7.0. When the procedure is run with 10 μL of the standard solution, the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Bleomycin Hydrochloride



Bleomycinoic acid: R = OH
Bleomycin A_1: R = $NHCH_2CH_2CH_2SOCH_3$
Bleomycin demethyl A_2: R = $NHCH_2CH_2CH_2SCH_3$
Bleomycin A_2: R = $NHCH_2CH_2CH_2S^+(CH_3)_2 \cdot X^-$
Bleomycin $A_{2'\text{-a}}$: R = $NHCH_2CH_2CH_2CH_2NH_2$
Bleomycin $A_{2'\text{-b}}$: R = $NHCH_2CH_2CH_2NH_2$
Bleomycin A_5: R = $NH(CH_2)_3NH(CH_2)_4NH_2$
Bleomycin $B_{1'}$: R = NH_2
Bleomycin B_2: R = $NH(CH_2)_4NHC(NH)NH_2$
Bleomycin B_4: R = $NH(CH_2)_4NHC(NH)NH(CH_2)_4NHC(NH)NH_2$

Bleomycinoic acid:
1-Bleomycinoic acid hydrochloride
Bleomycin A_1:
N^1-[3-(Methylsulfinyl)propyl]bleomycinamide hydrochloride
Bleomycin demethyl A_2:
N^1-[3-(Methylsulfanyl)propyl]bleomycinamide hydrochloride
Bleomycin A_2:
N^1-[3-(Dimethylsulfonio)propyl]bleomycinamide hydrochloride
Bleomycin $A_{2'\text{-a}}$:
N^1-(4-Aminobutyl)bleomycinamide hydrochloride
Bleomycin $A_{2'\text{-b}}$:
N^1-(3-Aminopropyl)bleomycinamide hydrochloride
Bleomycin A_5:
N^1-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide hydrochloride
Bleomycin $B_{1'}$:
Bleomycinamide hydrochloride
Bleomycin B_2:
N^1-(4-Guanidinobutyl)bleomycinamide hydrochloride
Bleomycin B_4:
N^1-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide hydrochloride
[*11066-06-7*, Bleomycin]

Bleomycin Hydrochloride is the hydrochloride of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.

Bleomycin Hydrochloride contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg of bleomycin A_2 ($C_{55}H_{84}N_{17}O_{21}S_3$: 1451.00), calculated on the dried basis.

Description Bleomycin Hydrochloride appears as white to yellowish white powder.
Bleomycin Hydrochloride is freely soluble in water and slightly soluble in ethanol (95).
Bleomycin Hydrochloride is hygroscopic.

Identification (1) To 4 mg each of Bleomycin Hydrochloride and Bleomycin A_2 Hydrochloride RS add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bleomycin Hydrochloride and Bleomycin A_2 Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

pH The pH of a solution obtained by dissolving 0.10 g of Bleomycin Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Content Ratio Dissolve 10 mg of Bleomycin Hydrochloride in 20 mL of water, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A_2 (the first principal peak) is between 55 % and 70 %, that of bleomycin B_2 (the second principal peak) is between 25 % and 32 %, the total peak area of bleomycin A_2 and bleomycin B_2 is not less than 85 %, the peak area of demethylbleomycin A_2 (a peak having the relative retention time of 1.5 to 2.5 to bleomycin A_2) is not more than 5.5 %, and the total area of the rest of the peaks is not more than 9.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9 : 1)

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3 : 2)

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediaminetetraacetate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-60	100→0	0→100
60-75	0	100

Flow rate: About 1.2 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the test solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0 %.

Time span of measurement: 20 minutes after elution of the peak of demethylbleomycin A_2 beginning after the solvent peak.

Purity (1) ***Clarity and color of solution***—A solution obtained by dissolving 80 mg of Bleomycin Hydrochloride in 4 mL of water is clear and colorless.

(2) ***Copper***—Dissolve exactly 75 mg of Bleomycin Hydrochloride in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the test solution. Separately, to exactly 15 mL of standard copper solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the test solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Dissolved acetylene – Air
Lamp: Copper hollow cathode lamp
Wavelength: 324.8 nm

Loss on Drying Not more than 5.0 % (60 mg, in vacuum, P_2O_5, 60 °C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

Sterility Test It meets the requirement.

Pyrogen Test It meets the requirement, when Bleomycin Hydrochloride is used in a sterile preparation. Weigh a suitable amount of Bleomycin Hydrochloride, make a solution so that each mL contains 200 μg (potency), and use this solution as the test solution. Inject into rabbits 1.0 mL of the test solution per kg.

Histamine It meets the requirement, when Bleomycin Hydrochloride is used in a sterile preparation. Weigh a suitable amount of Bleomycin Hydrochloride, make a solution so that each mL contains 300 μg (potency), and use this solution as the test solution.

Assay ***The Cylinder-plate method*** (1) Culture medium (i) Agar media for seed, base layer and transferring the test organism-

Glycerin	10.0 g
Peptone	10.0 g
Sodium chloride	3.0 g
Meat extract	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with sodium hydroxide TS so that it will be between 6.9 and 7.1 after sterilization.

(ii) Liquid medium for suspending the test organism-

Glycerin	10.0 g
Peptone	10.0 g
Sodium chloride	3.0 g
Meat extract	10.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with sodium hydroxide TS so that it will be between 6.9 and 7.1 after sterilization.

(iii) Preparation of seeded agar layer- Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27 °C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid medium for suspending the test organism, cultivate with shaking at 25 to 27 °C for 5 days, and use this as the test organism suspension. Store the test organism suspension at a temperature not exceeding 5 °C, and use within 14 days. Add 0.5 mL of the test organism suspension to 100 mL of the agar medium for seed previously kept at 48 °C, mix thoroughly, and use as the seeded agar layer.

(2) Test organism- *Mycobacterium smegmatis* ATCC 607

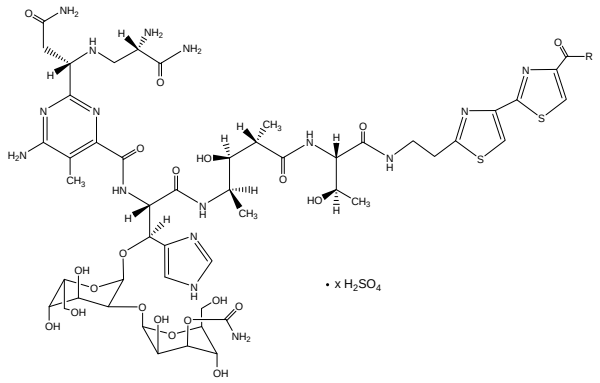
(3) Preparation of cyinder-agar plate- Proceed as directed in 7. Preparation of Cylinder-agar Plates, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(4) Weigh accurately an amount of Bleomycin Hydrochloride, equivalent to about 15 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately an amount of Bleomycin A_2 Hydrochloride RS, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve

in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL, and use this solution as the standard stock solution. Pipet a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration stock solution and low concentration stock solution, respectively. Perform the test with these solutions as directed in I) (8) under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Bleomycin Sulfate



Bleomycinoic acid: R = OH
Bleomycin A_1: R = $NHCH_2CH_2CH_2SOCH_3$
Bleomycin demethyl A_2: R = $NHCH_2CH_2CH_2SCH_3$
Bleomycin A_2: R = $NHCH_2CH_2CH_2S^+(CH_3)_2 \cdot X^-$
Bleomycin $A_{2'\text{-a}}$: R = $NHCH_2CH_2CH_2CH_2NH_2$
Bleomycin $A_{2'\text{-b}}$: R = $NHCH_2CH_2CH_2NH_2$
Bleomycin A_5: R = $NH(CH_2)_3NH(CH_2)_4NH_2$
Bleomycin $B_{1'}$: R = NH_2
Bleomycin B_2: R = $NH(CH_2)_4NHC(NH)NH_2$
Bleomycin B_4: R = $NH(CH_2)_4NHC(NH)NH(CH_2)_4NHC(NH)NH_2$

Bleomycinoic acid:
1-Bleomycinoic acid sulfate
Bleomycin A_1:
N^1-[3-(Methylsulfinyl)propyl]bleomycinamide sulfate
Bleomycin Demethyl A_2:
N^1-[3-(Methylsulfanyl)propyl]bleomycinamide sulfate
Bleomycin A_2:
N^1-[3-(Dimethylsulfonio)propyl]bleomycinamide sulfate
Bleomycin $A_{2'\text{-a}}$:
N^1-(4-Aminobutyl)bleomycinamide sulfate
Bleomycin $A_{2'\text{-b}}$:
N^1-(3-Aminopropyl)bleomycinamide sulfate
Bleomycin A_5:
N^1-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide sulfate
Bleomycin $B_{1'}$:
Bleomycinamide sulfate
Bleomycin B_2:
N^1-(4-Guanidinobutyl)bleomycinamide sulfate
Bleomycin B_4:
N^1-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide sulfate
[*9041-93-4*]

Bleomycin Sulfate is the sulfate of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.
Bleomycin Sulfate contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg of bleomycin A_2 ($C_{55}H_{84}ClN_{17}O_{21}S_3$: 1451.01), calculated on the dried basis.

Description Bleomycin Sulfate is a white to yellowish white powder.
Bleomycin Sulfate is freely soluble in water and slightly soluble in ethanol (95).
Bleomycin Sulfate is hygroscopic.

Identification (1) To 4 mg each of Bleomycin Sulfate and Bleomycin Sulfate RS add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bleomycin Sulfate and Bleomycin Sulfate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Sulfate (1 in 200) responds to the Qualitative Tests (1) and (2) for sulfate.

pH The pH of a solution obtained by dissolving 10 mg of Bleomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity (1) ***Clarity and color of solution***—A solution obtained by dissolving 80 mg of Bleomycin Sulfate in 4 mL of water is clear and colorless.

(2) ***Copper***—Dissolve exactly 75 mg of Bleomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the test solution. Separately, pipet 15 mL of standard copper solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the test solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Dissolved acetylene – Air

Lamp: Copper hollow cathode lamp
Wavelength: 324.8 nm

Loss on Drying Not more than 5.0 % (60 mg, in vacuum, P_2O_5, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Bleomycin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 10 EU/mg (potency) of bleomycin, when Bleomycin Sulfate is used in a sterile preparation.

Histamine It meets the requirement, when Bleomycin Sulfate is used in a sterile preparation. Weigh appropriate amount of Bleomycin Sulfate, dissolve in water, make the solution so that each mL contains 300 μg (potency), and use the solution as the test solution.

Content Ratio of Bleomycin Weigh accurately 10 mg (potency) of Bleomycin Sulfate, dissolve in 20 mL of water, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions, and determine each peak area. Based on the % of peak area, bleomycin A_2 (the first principal peak) is between 55 % and 70 %, bleomycin B_2 (the second principal peak) is between 25 % and 32 %, the total peak area of bleomycin A_2 and bleomycin B_2 is not less than 85 %, the peak area of dimethylbleomycin A_2 (a peak having the relative retention time of 1.5 ~ 2.5 against bleomycin A_2) is not more than 5.5 %, and the total area of the rest peaks is not more than 9.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter)

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Mobile phase A : A mixture of the mobile phase stock solution and methanol (9:1)

Mobile phase B : A mixture of the mobile phase stock solution and methanol (3:2)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-60	100→0	0→100
60-75	0	100

Flow rate: About1.2 mL per min
System suitability

System performance: When the procedure is run with 20 μL of the test solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0 %.

Time span of measurement: Twenty minutes after elution of the peak of demethylbleomycin A_2 beginning after the solvent peak.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate in 1000 mL of water and 5 mL of glacial acetic acid, and adjust the pH to 4.3 with ammonia TS.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer-

Peptone	10.0 g	Glycerine	10.0 g
Meat extract	10.0 g	Agar	15.0 g
Sodium chloride	1.5 g	Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution with sodium chloride TS so that it will be 6.9 to 7.1 after sterilization.

(2) Liquid media for suspending the test organism

Peptone	10.0 g	Sodium chloride	3.0 g
Meat extract	10.0 g	Glycerine	10.0 g
Water	1000 mL		

Mix all the ingredients, and sterilize. Adjust the pH of the solution with sodium chloride TS so that it will be 6.9 to 7.1 after sterilization.

(3) Test organism - *Mycobacterium smegmatis* ATCC 607.

(4) Preparation of seeded agar layer – Cultivate the test organism, on the slant of the agar medium for transferring the test organism at 27 °C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25 °C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5 °C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48 °C, mix thoroughly, and use as the seeded agar layer.

(5) Agar plate – Proceed as directed in I 5 under the Microbial Assay for Antibiotics. Use 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed.

(6) Weigh accurately about 5 mg (potency) of Bleomycin Sulfate, dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make the solutions so that each mL contains 30.0 μg (potency) and 15.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution. Separately, weigh accurately an amount of Bleomycin A_2 Hydrochloride RS, previously dried,

equivalent to about 5 mg (potency) and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make the solution so that each mL contains 150.0 μg (potency) and use the solution as the standard stock solution. Keep the standard solution at not exceeding 5 °C and use within 30 days. Take exactly a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30.0 μg (potency) and 15.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Boric Acid

H_3BO_3: 61.83

[*10043-35-3*]

Boric Acid, when dried, contains not less than 99.5 % and not more than 101.0 % of boric acid (H_3BO_3).

Description Boric Acid appears as colorless or white crystals or crystalline powder, odorless and has a slight, characteristic taste.
Boric Acid is freely soluble in warm water, in hot ethanol or in glycerin, soluble in water or in ethanol (95) and practically insoluble in ether.

pH — A solution of Boric Acid (1 in 20) is between 3.5 and 4.1.

Identification A solution of Boric acid (1 in 20) responds to the Qualitative Tests for borate.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Boric acid in 25 mL of water or in 10 mL of hot ethanol: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Boric Acid according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 0.40 g of Boric Acid according to Method 1 and perform the test (not more than 5 ppm).

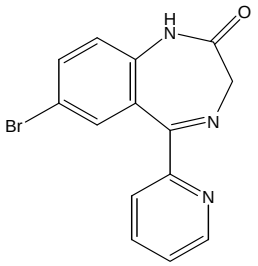
Loss on Drying Not more than 0.5 % (2 g, silica gel, 5 hours).

Assay Weigh accurately 1.5 g of Boric Acid, previously dried, add 15 g of D-Sorbitol and 50 mL of water, ant dissolve by warming. After cooling, titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 61.83 mg of H_3BO_3

Containers and Storage ***Containers***—Well-closed containers.

Bromazepam



$C_{14}H_{10}BrN_3O$: 316.15

7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-1,4-benzodiazepin-2-one [*1812-30-2*]

Bromazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of bromazepam ($C_{14}H_{10}BrN_3O$).

Description Bromazepam appears as white to pale yellowish white crystals or crystalline powder and is odorless.
Bromazepam is freely soluble in *N,N*-dimethylformamide or in acetic acid (100), sparingly soluble in chloroform, slightly soluble in methanol or in ethanol (99.5), very slightly soluble in ether and practically insoluble in water.
Bromazepam dissolves in dilute hydrochloric acid.

Melting point— About 245 ºC (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Bromazepam and Bromazepam RS in ethanol (99.5) (1 in 200000) under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the absorption spectra of Bromazepam and Bromazepam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelnumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Bromazepam in a platinum crucible according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 50 mg of Bromazepam in 5 mL of a mixture of acetone and methanol (3 : 2) and use this solution as the test solu-

tion. Pipet 1 mL of the test solution and add a mixture of acetone and methanol (3 : 2) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of acetone and methanol (3 : 2) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (38 : 1 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution and the spot of the starting point are not more than 2 and not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, 105 ºC, 4 hours).

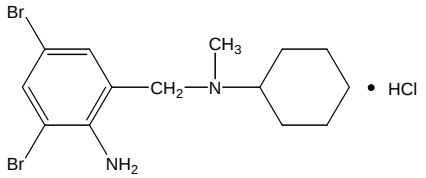
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.4 g of Bromazepam, previously dried, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.615 mg of $C_{14}H_{10}BrN_3O$

Containers and Storage ***Containers***—Well-closed containers.

Bromhexine Hydrochloride



$C_{14}H_{20}Br_2N_2 \cdot HCl$: 412.59

2,4-Dibromo-6-
{[cyclohexyl(methyl)amino]methyl}aniline
hydrochloride [*611-75-6*]

Bromhexine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of bromhexine ($C_{14}H_{20}Br_2N_2 \cdot HCl$).

Description Bromhexine Hydrochloride appears as white crystals or crystalline powder, is odorless and tasteless.
Bromhexine Hydrochloride is freely soluble in formic acid, sparingly soluble in methanol and slightly soluble in water or ethanol (95).

pH— The pH of saturated solution of Bromhexine Hydrochloride is between 3.0 and 5.0.

Melting point— About 239 ºC (with decomposition).

Identification (1) Dissolve 3 mg each of Bromhexine Hydrochloride and Bromhexine Hydrochloride RS in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Bromhexine Hydrochloride and Bromhexine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Add 20mL of water to 1 g of Brohexine Hydrochloride. After thorough shaking, add 3 mL of sodium hydroxide TS and extract with four 20 mL portions of ether. Neutralize the water layer with dilute nitric acid: the solution responds to the Qualitatve Test (2) for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Bromhexine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Perform this test without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Bromhexine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL and this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine each peak area by the automatic integration method: each peak area other than bromhexine from the test solution is not larger than the peak area of bromhexine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 245 nm)

Column: A stainless steel column about 5 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC

Mobile phase: Dissolve 1.0 g of potassium hydrogen phosphate in 900 mL of water, adjust the pH to 7.0 with 0.5 mol/L sodium hydroxide TS and add water to make 1000 mL. To 200 mL of this solution 800 mL of acetonitrile.

Flow rate: Adjust flow rate so that the retention time of bromhexine is about 6 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bromhexine from 5 μL of the standard solution is between 5 mm and 15 mm.

System performance: To 50 mg of bamethane sulfate add 0.5 mL of the test solution and add the mobile phase to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, bamethane and bromhexine are eluted in this order with the resolution between these peaks being not less than 7.

Time span of measurement: About 2 times as long as the retention time of bromhexine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

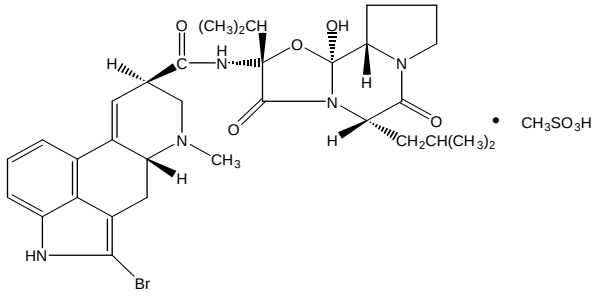
Assay Weigh accurately 0.5 g of Bromhexine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride and warm in a 50 ºC water-bath and cool. Titrate with 0.1 mol/L perchloric acid until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.26 mg of $C_{14}H_{20}Br_2N_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Bromocriptine Mesilate



$C_{32}H_{40}BrN_5O_5 \cdot CH_4O_3S$: 750.70

(5'*S*)-2-Bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6',18-trione; monomethanesulfonic acid [*22260-51-1*]

Bromocriptine Mesilate contains not less than 98.0 % and not more than 101.0 % of bromocriptine mesilate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4O_3S$), calculated on the dried basis.

Description Bromocriptine Mesilate is a white to pale yellowish white or pale brownish white, crystalline powder and is odorless or has a faint characteristic odor.

Bromocriptine Mesilate is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in acetic anhydride, in dichloromethane or in chloroform and practically insoluble in water or in ether.

Bromocriptine Mesilate is gradually affected by light.

Identification (1) Dissolve 2 mg of Bromocriptine Mesilate in 1 mL of methanol, add 2 mL of *p*-dimethylaminobenzaldehyde-iron (III) chloride TS and shake: a purplish blue color is observed.

(2) Determine the absorption spectra of the solutions of Bromocriptine Mesilate and Bromocriptine Mesilate RS in methanol (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorptioni at the same wavelengths.

(3) Determine the infrared spectra of Bromocriptine Mesilate and Bromocriptine Mesilate RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Bromocriptine Mesilate as directed under the Flame Coloration Test (2): a green color is observed.

Specific Optical Rotation $[\alpha]_D^{20}$: +95 ~ +105° [0.1 g previously dried, a mixture of methanol and dichloromethane (1 : 1), 10 mL, 100 mm].

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of methanol: the solution is clear and has no more color than the following control solution.

Control solution—To 2.5 mL of cobalt (II) chloride hexahydrate stock CS, 6.0 mL of iron (III) chloride hexahydrate stock CS and 1.0 mL of cupric sulfate stock CS, add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) ***Heavy metals***—Proceed with 1.0 g of Bromocriptine Mesilate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform this test without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of a mixture of methanol and chloroform (1 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of methanol and chloroform (1 : 1) to make exactly 200 mL and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of methanol and chloroform (1 : 1) to make exactly 20 mL and use this solution as the standard solution (2). Perform the test with the test

solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2), as a band with 1 cm in width, on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate immediately with a mixture of dichloromethane, 1,4-dioxane, ethanol (95) and ammonia solution (28) (1800 : 150 : 50 : 1) to a distance of about 10 cm and dry the plate under reduced pressure for 30 minutes. Spray evenly Dragendorff's TS on the plate, then spray evenly hydrogen peroxide TS, cover the plate with a glass plate and examine: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (1) and the spot other than the principal spot, which is more intense than the spot from the standard solution (2), is not more than one.

Loss on Drying Not more than 3.0 % (1 g, not exceeding 0.67 kPa, 80 ºC, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Mesilate Content Weigh accurately 0.4 g of Bromocriptine Mesilate, dissolve in 70 mL of methanol, and titrate with 0.1 mol/L potassium hydroxide-methanol TS (potentiometric titration, Endpoint Detection Method in Titrimetry) with the aid of a current of nitrogen. Perform a blank determination and make any necessary correction. The content of mesilate is not less than 12.5 % and not more than 13.4 %, calculated on the dried basis.

Each mL of 0.1 mol/L
potassium hydroxide-methanol TS
= 9.61 mg of CH_3SO_3H

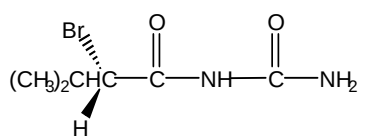
Assay Weigh accurately 0.6 g of Bromocriptine Mesilate, dissolve in 80 mL of a mixture of acetic acid (100) and acetic anhydride (7 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 75.07 mg of $C_{32}H_{40}BrN_5O_5 \cdot CH_4O_3S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding −18 ºC.

Bromvalerylurea



and enantiomer

$C_6H_{11}BrN_2O_2$: 223.07

2-Bromo-*N*-carbamoyl-3-methylbutanamide [*496-67-3*]

Bromovalerylurea, when dried, contains not less than 98.0 % and not more than 101.0 % of bromovalerylurea ($C_6H_{11}BrN_2O_2$).

Description Bromovalerylurea appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste.
Bromovalerylurea is soluble in ethanol (95), sparingly soluble in ether and very slightly soluble in water.
Bromovalerylurea dissolves in sulfuric acid, nitric acid or hydrochloric acid and precipitates are produced on the addition of water.
Bromovalerylurea dissolve in sodium hydroxide TS.

Identification (1) Boil 0.2 g of Bromovalerylurea with 5 mL of a solution of sodium hydroxide (1 in 10): the gas evolved changes moistened red litmus paper to blue. Boil this solution with an excess of dilute sulfuric acid: the odor of valeric acid is perceptible,

(2) To 0.1 g of Bromovalerylurea, add 0.5 g of anhydrous sodium carbonate and decompose thoroughly by gentle heating. Dissolve the residue in 5 mL of hot water, cool, acidify with acetic acid and filter: the filtrate responds to the Qualitative Tests (2) for bromide.

Melting Point 151 ~ 155 ºC.

Purity (1) ***Acidity or alkalinity***—To 1.5 g of Bromovalerylurea, add 30 mL of water, shake for 5 minutes and filter: the filtrate is neutral.

(2) ***Chloride***—Perform the test with a 10 mL of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028 %).

(3) ***Sulfate***—Perform the test with 10 mL of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Bromovalerylurea according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Dissolve 0.5 g of Bromovalerylurea in 5 mL of sodium hydroxide TS, use this solution as the test solution and perform the test (not more than 4 ppm).

(6) ***Readily carbonizable substances***—Perform the test with 0.5 g of Bromovalerylurea: the solution has no more color than Color Matching Fluid A.

Loss on Drying Not more than 0.5 % (1 g, 80 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

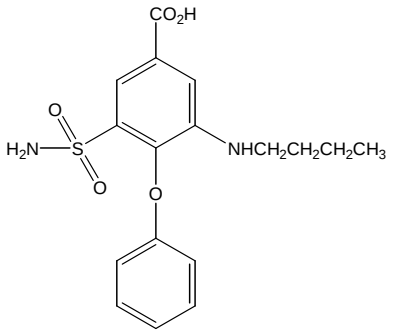
Assay Weigh accurately 0.4 g of Bromovalerylurea,

previously dried, in an Erlenmeyer flask, add 40 mL of sodium hydroxide TS and boil gently for 20 minutes under a reflux condenser. Cool, wash the lower part of the reflux condenser and the mouth of the flask with 30 mL of water and combine the washings with the solution in the Erlenmeyer flask. Add 5 mL of nitric acid and exactly 30 mL of 0.1 mol/L silver nitrate VS and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 22.307 mg of $C_6H_{11}BrN_2O_2$

Containers and Storage ***Containers***—Well-closed containers.

Bumetanide



$C_{17}H_{20}N_2O_5S$: 364.42

3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid
[*28395-03-1*]

Bumetanide, when dried, contains not less than 98.5 % and not more than 101.0 % of bumetanide ($C_{17}H_{20}N_2O_5S$).

Description Bumetanide appears as white crystals or crystalline powder.
Bumetanide is freely soluble in pyridine, soluble in methanol and in ethanol (95), slightly soluble in ether and practically insoluble in water.
Bumetanide dissolves in potassium hydroxide TS.
Bumetanide is gradually colored by light.

Identification (1) Dissolve 10 mg of Bumetanide in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, shake, add 3 mL of water and 5 mL of chloroform, shake and allow to stand: a pale blue color develops in the chloroform layer.

(2) Dissolve 40 mg each of Bumetanide and Bumetanide RS in 100 mL of phosphate buffer solution, pH 7.0. To 10 mL of these solutions, add water to make 100 mL and determine their absorption spectra as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Bumetanide and Bumetanide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 232 ~ 237 ºC

Purity (1) ***Clarity and color of solution***— Dissolve 50 mg of Bumetanide in 2 mL of a solution of potassium hydroxide (1 in 30) and 8 mL of water: the solution is clear and has no more color than the following control solution.

Control solutions—Pipet 0.5 mL each of cobalt (II) chloride hexahydrate stock CS, iron (III) chloride hexahydrate stock CS and cupric sulfate stock CS, mix and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) ***Chloride*** —Mix well 0.5 g of Bumetanide with 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, transfer, in small portions, to a red-hot platinum crucible and heat until the reaction is complete. After cooling, to the residue, add 14 mL of dilute sulfuric acid and 6 mL of water, boil for 5 minutes, filter, wash the residue, with 10 mL of water, combine the filtrate and the washing and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Bumetanide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Bumetanide according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Perform this test without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bumetanide in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetic acid (100), cyclohexane and methanol (32 : 4 : 4 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

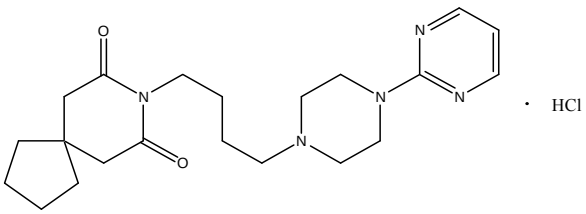
Assay Weigh accurately about 0.5 g of Bumetanide, previously dried, dissolve in 50 mL of ethanol (95) and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry), Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.442 mg of $C_{17}H_{20}N_2O_5S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Buspirone Hydrochloride



$C_{21}H_{31}N_5O_2 \cdot HCl$: 421.96

8-[4-(4-Pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride
[*33386-08-2*]

Buspirone Hydrochloride contains not less than 97.5 % and not more than 102.5 % of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$), calculated on the anhydrous basis.

Description Buspirone Hydrochloride is a white, crystalline powder.
Buspirone Hydrochloride is very soluble in water, freely soluble in methanol or in dichlormethane, sparingly soluble in ethanol (95) or in acetonitrile, very slightly soluble in ethyl acetate, and practically insoluble in hexane.

Identification (1) Determine the infrared spectra of Buspirone Hydrochloride and Buspirone Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When the procedure in the Assay is performed, the relative retention time of the principal peak from the test solution corresponds to that from the standard solution.

(3) A solution of Buspirone Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Purity ***Heavy metals***—Proceed with 1.0 g of Buspirone Hydrochloride, according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (20 ppm).

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Dissolve about 50 mg each of Buspirone Hydrochloride and Buspirone Hydrochloride RS, accurately weighed, in 1 mol/L to make 25 mL, dilute with water to make 100 mL. To 10 mL of these solutions, add 10 mL of the internal standard, and dilute with water to make 50 mL. Use these solutions as the test solution and the standard solution, respectively. Perform the test with 25 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of Buspirone Hydrochloride.

Amount (mg) of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$)
= Amount (mg) of Buspirone Hydrochloride RS

$$\times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 0.25 g of propyl parahydroxybenzoate in methanol to make 100 mL. Dilute 25 mL of this solution with water to 500 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.0 mm internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer solution and acetonitrile (60:40).

Flow rate: 2 mL/min.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution according to the above operating conditions, the resolution between the peaks of buspirone hydrochloride and the internal standard is not less than 4.0.

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of buspirone hydrochloride to that of the internal standard is not more than 2.0.

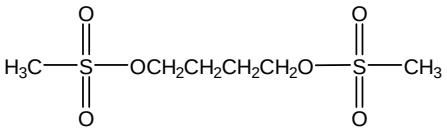
Buffer solution—Dissolve 1.36 g of monobasic po-

tassium phosphate in water to make 1000 mL, and adjust the solution with 10 % sodium hydroxide to a pH of 7.5.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Busulfan



$C_6H_{14}O_6S_2$: 246.30

4-Methylsulfonyloxybutyl methanesulfonate [*55-98-1*]

Busulfan contains not less than 98.5 % and not more than 101.0 % of busulfan ($C_6H_{14}O_6S_2$), calculated on the dried basis.

Description Busulfan is a white, crystalline powder and is odorless.
Busulfan is slightly soluble in ether, very slightly soluble in ethanol (95) and practically insoluble in water.

Identification (1) To 0.1 g of Busulfan, add 10 mL of water and 5 mL of sodium hydroxide TS, dissolve by heating and use this solution as the test solution. (i) To 7 mL of the test solution, add 1 drop of potassium permanganate TS: the red-purple color of potassium permanganate TS changes from blue-purple trough blue to green. (ii) Acidify 7 mL of the test solution with dilute sulfuric acid and add 1 drop of potassium permanganate TS: the color of potassium permanganate TS remains.

(2) Determine the infrared spectra of Busulfan and Busulfan RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 115 ~ 118 ºC

Purity (1) ***Sulfate***—To 1.0 g of Busulfan, add 40 mL of water and dissolve by heating. Cool in ice for 15 minutes and filter. Wash the residue with 5 mL of water, combine the washings with the filtrate and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Busulfan according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 2.0 % (1 g, in vacuum, P_2O_5, 60 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Busulfan, add 40 mL of water and boil gently under a reflux condenser for 30 minutes. Cool and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.315 mg of $C_6H_{14}O_6S_2$

Containers and Storage ***Containers***—Well closed containers.

Storage—Light-resistant.

Busulfan Tablets

Busulfan Tablets contains not less than 93.0 % and not more than 107.0 % of the labeled amount of busulfan ($C_6H_{14}O_6S_2$: 246.30).

Method of Preparation Prepare as directed under Tablets, with Busulfan.

Identification Powder Busulfan Tablets and extract the powder several times with acetone. Collect these extracts and evaporate to dryness in a water-bath, with the aid of a current of air. The residue responds to Identification test (1) and (2) of Busulfan, and melts at about 115 ºC.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh and finely powder not less than 40 tablets (caution: guard against accidental inhalation of fine powder). Weigh accurately a portion of powder, equivalent to about 80 mg of busulfan ($C_6H_{14}O_6S_2$) and transfer to a beaker. Extract with four 20 mL volumes of acetone, each time stirring the mixture well, then allowing the insoluble matter to settle and finally decanting the clear supernatant liquid through a sintered-glass filter (G4). Evaporate the collection of extracts to about 10 mL, add phenolphthalein TS and neutralize with 0.1 mol/L sodium hydroxide. Evaporate to dryness, add about 30 mL of water and proceed as directed under the Assay of Busulfan with reflux condenser (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.315 mg of $C_6H_{14}O_6S_2$

Containers and Storage ***Containers***—Well closed containers.

Storage—Light-resistant.

Caffeine and Sodium Benzoate

Caffeine and Sodium Benzoate, when dried, contains not less than 48.0 % and not more than 50.0 % of caffeine ($C_8H_{10}N_4O_2$: 194.19) and not less than 50.0 % and not more than 52.0 % of sodium benzoate ($C_7H_5NaO_2$: 144.10).

Description Caffeine and Sodium Benzoate is a white powder, is odorless and has a slightly bitter taste. Caffeine and Sodium Benzoate is freely soluble in water, soluble in acetic acid (100) or in acetic anhydride, sparingly soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Dissolve 1 g of Caffeine and Sodium Benzoate in 10 mL of water in a separator, add 1 drop of phenolphthalein TS and add carefully 0.01 mol/L sodium hydroxide VS dropwise until a pale red color develops. Extract with three 20 mL volume of chloroform by thorough shaking and separate the chloroform layer from the water layer [use the water layer for test (2).]. Filter the combined chloroform extracts, evaporate the filtrate to dryness in a water-bath and proceed the following tests with the residue. (i) To 2 mL of a solution of the residue (1 in 500), add tannic acid TS drop-wise: a white precipitate, which dissolves upon the drop-wise addition of tannic acid TS, is produced. (ii) To 10 mg of the residue, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, evaporate to dryness on a water-bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS. (iii) Dissolve 10 mg of the residue in water to make 50 mL. To 5 mL of this solution, add 3 mL of diluted acetic acid (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5) and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

(2) To 5 mL of the water layer obtained in (1), add 5 mL of water: the solution responds to the Qualitative Tests (2) for benzoate.

(3) Heat Caffeine and Sodium Benzoate: white fumes are evolved. Ignite furthermore and to the residue, add hydrochloric acid: bubbles are produced and the solution responds to the Qualitative Tests (1) for sodium salt.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Caffeine and Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) ***Alkali***—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 20 mL of water and add 1 or 2 drops of phenolphthalein TS: no red color develops.

(3) ***Chloride***—Dissolve 0.5 g of Caffeine and Sodium Benzoate in 10 mL of water and add 30 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95) and water to make 50 mL (not more than 0.050 %).

(4) ***Chlorinated compounds***—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 40 mL of water, add 10 mL of dilute sulfuric acid and extract with two 2 mL volumes of ether. Allow the combined ether extracts to evaporate at room temperature to dryness. Place this residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water and dry. Ignite at about 600 ºC, dissolve the residue in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings and add water to make 50 mL. To this solution, add 0.5 mL of silver nitrate TS: the solution shows no more turbidity than the following control solution to which 0.5 mL of silver nitrate TS has been added.

Control solution—Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings and add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL.

(5) ***Heavy metals***—Dissolve 2.0 g of Caffeine and Sodium Benzoate in 47 mL of water, add slowly, with vigorous stirring, 3 mL of dilute hydrochloric acid and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL of the filtrate with ammonia TS and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) ***Arsenic***—Prepare the test solution with 1.0 g of Caffeine and Sodium Benzoate according to Method 1 and perform the test (not more than 2 ppm).

(7) ***Phthalic acid***— Dissolve exactly about 100 mg of Caffeine and Sodium Benzoate in 1 mL of water, add 1 mL of resorcinol-sulfuric acid TS, and heat in an oil bath at a temperature between 120 and 125 °C. After evaporating the water, heat again for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of this solution add 10 mL of a solution of sodium hydroxide (43 in 500), and use this solution as the test solution. Separately, dissolve exactly 61 mg of potassium biphthalate in 1000 mL of water. Proceed with 1 mL of this solution in the same manner as the test solution, and use this solution as the standard solution. Determine the absorbances at 495 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution is not more than that of the standard solution.

(8) ***Readily carbonizable substances***— Proceed

with 0.5 g of Caffeine and Sodium Benzoate and perform the test: the solution has no more color than Color Matching Fluid A.

Loss on Drying Not more than 3.0 % (2 g, 80 ºC, 4 hours).

Assay (1) ***Sodium benzoate***—Weigh accurately 0.2 g of Caffeine and Sodium Benzoate, previously dried, dissolve by warming in 50 mL of a mixture of acetic anhydride and acetic acid (100) (6 : 1), cool and titrate with 0.1 mol/L perchloric acid-1,4-dioxane VS to the first equivalence point (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

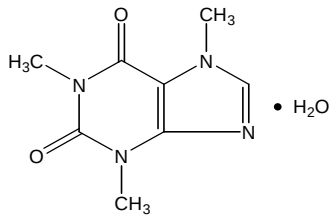
Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
= 14.411 mg of $C_7H_5NaO_2$

(2) ***Caffeine***—Continue the titration in (1) with 0.1 mol/L perchloric acid-1,4-dioxane VS from the first equivalence point to the second equivalence point (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
= 19.419 mg of $C_8H_{10}N_4O_2$

Containers and Storage ***Containers***—Well-closed containers.

Caffeine Hydrate



$C_8H_{10}N_4O_2 \cdot H_2O$: 212.21

1,3,7-Trimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione hydrate [*5743-12-4*]

Caffeine Hydrate, previously dried, contains not less than 98.5 % and not more than 101.0 % of caffeine ($C_8H_{10}N_4O_2$: 194.19).

Description Caffeine Hydrate appears as white, soft crystals or powder, is odorless and has a slightly bitter taste.
Caffeine Hydrate is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) or in acetic anhydride, slightly soluble in ethanol (95) and very slightly soluble in ether.

pH — A solution of Caffeine Hydrate (1 in 100) is between 5.5 and 6.5.

Caffeine Hydrate effloresces in dry air.

Identification (1) To 2 mL of a solution of Caffeine Hydrate (1 in 500), add tannic acid TS drop-wise: a white precipitate, which dissolves upon the drop-wise addition of tannic acid TS, is produced.

(2) To 10 mg of Caffeine Hydrate, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid and evaporate to dryness on a water-bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 10 mg of Caffeine Hydrate in water to make 50 mL. To 5 mL of this solution, add 3 mL of diluted acetic acid (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5) and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting Point 235 ~ 238 ºC (after drying).

Purity (1) ***Chloride***—Dissolve 2.0 g of Caffeine Hydrate in 80 mL of hot water, cool rapidly to 20 ºC, add water to make 100 mL and use this solution as the test stock solution. To 40 mL of the test stock solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(2) ***Sulfate***—To 40 mL of the test stock solution obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Caffeine Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Caffeine Hydrate in 10 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

(5) ***Readily carbonizable substance***—Perform the test using 0.5 g of Caffeine Hydrate: the solution has

no more color than Color Matching Fluid D.

Loss on Drying 0.5 ~ 8.5 % (1 g, 80 ºC, 4 hours).

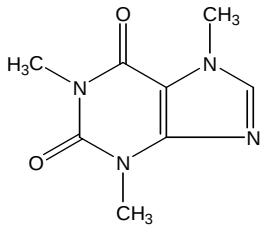
Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.4 g of Caffeine Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6 : 1) and titrate with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.419 mg of $C_8H_{10}N_4O_2$

Containers and Storage ***Containers***—Tight containers.

Anhydrous Caffeine



Caffeine $C_8H_{10}N_4O_2$: 194.19

1,3,7-Trimethylpurine-2,6-dione [*58-08-2*]

Caffeine, when dried, contains not less than 98.5 % and not more than 101.0 % of caffeine ($C_8H_{10}N_4O_2$).

Description Caffeine appears as white crystals or powder, is odorless and has a bitter taste.
Caffeine is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) or in acetic anhydride and slightly soluble in ethanol (95) or in ether.

pH— A solution of Caffeine (1 in 100) is between 5.5 and 6.5

Identification (1) To 2 mL of a solution of Caffeine (1 in 500), add tannic acid TS drop-wise: a white precipitate, which dissolves upon the drop-wise addition of tannic acid TS, is produced.

(2) To 10 mg of Caffeine, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid and evaporate on a water-bath to dryness: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns a red-purple and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 10 mg of Caffeine in water to make 50 mL. To 5 mL of this solution, add 3 mL of diluted acetic acid (3 in 100) and 5 mL of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5) and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting Point 235 ~ 238 ºC.

Purity (1) ***Chloride***—Dissolve 2.0 g of Caffeine in 80 mL of hot water, cool rapidly to 20 ºC, add water to make 100 mL and use this solution as the test stock solution. To 40 mL of the test stock solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(2) ***Sulfate***—To 40 mL of the test stock solution obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Caffeine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Caffeine in 10 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

(5) ***Readily carbonizable Substances***—Perform the test using 0.5 g of Caffeine: the solution has no more color than Color Matching Fluid D.

Loss on Drying Not more than 0.5 % (1 g, 80 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately 0.4 g of Caffeine, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6 : 1) and titrate with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of methyl-rosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.419 mg of $C_8H_{10}N_4O_2$

Containers and Storage ***Containers***—Tight containers.

Calamine

zinc; iron(3+); oxygen(2-) [*8011-96-9*]

Calamine is zinc oxide with a small proportion of ferric oxide. Calamine contains, after ignition, not less than 98.0 % and not more than 100.5 % of zinc oxide (ZnO: 81.37).

Description Calamine appears as pale red, fine powder, and is odorless and tasteless.
Calamine is practically insoluble in water.
Calamine dissolves in hydrochloric acid.

Identification (1) Dissolve 1 g of Calamine in 10 mL of 3 mol/L hydrochloric acid and filter: the filtrate responds to the Qualitative Tests for zinc.

(2) Treat 1 g of Calamine with 10 mL of 3 mol/L hydrochloric acid, heat to boiling and filter: the filtrate produces a red color upon the addition of ammonium thiocyanate TS.

Purity (1) ***Acid-insoluble substances***—Dissolve 2.0 g of Calamine in 50 mL of 3 mol/L hydrochloric acid. Filter of an insoluble residue remains. Wash with water, dry at 105 ºC for 1 hour, cool and weigh: the weight of the residue does not exceed 40 mg (not more than 2.0 %).

(2) ***Alkaline substances***—Add 1.0 g of Calamine to 20 mL of water in a water-bath for 15 minutes. Filter and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid: the color of the solution is colorless.

(3) ***Lead***—Add 15 mL of water to 1 g of Calamine, stir, then add 3 mL of acetic acid (100). Warm on a steam-bath until dissolved. Filter the mixture and add 5 drops of potassium chromate TS: no turbidity is produced.

(4) ***Calcium***—Dissolve 1 g of Calamine in 25 mL of 3 mol/L hydrochloric acid for 30 minutes and add 6 mol/L ammonium hydroxide to the filtrate until the precipitate first formed is dissolved, then add 5 mL more of 6 mol/L ammonium hydroxide. To 10 mL of this solution, add 2 mL of ammonium oxalate TS: not more than a slight turbidity is produced.

(5) ***Calcium or magnesium***—To another 10 mL portion of the solution prepared for the test for Calcium, add 2 mL of dibasic sodium phosphate TS: not more than a slight turbidity is produced.

(6) ***Arsenic***—Dissolve 0.25 g of Calamine in 35 mL of water and use this solution as the test solution and perform the test (not more than 8 ppm).

Loss on Ignition Not more than 2.0 % (2 g).

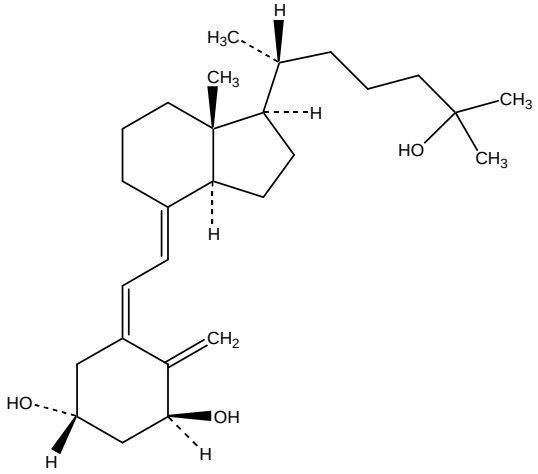
Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g and *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

Assay Dissolve about 1.5 g of freshly ignited Calamine, accurately weighed, with 50 mL of 0.5 mol/L sulfuric acid VS. Filter the mixture and wash the residue on the filter with hot water until last washing is neutral. To the combined filtrate and washings, add 2.5 g of the ammonium chloride, cool and titrate with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl orange TS). Perform a blank determination and make any necessary correction.

Each mL of 0.5 mol/L sulfuric acid = 40.69 mg of ZnO

Containers and Storage ***Containers***—Well-closed containers.

Calcitriol



$C_{27}H_{44}O_3$: 416.64

(1*R*,3*S*,5*Z*)-5-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-1-[(2*R*)-6-Hydroxy-6-methylheptan-2-yl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol [*32222-06-3*]

Calcitriol contains not less than 97.0 % and not more than 103.0 % of calcitriol ($C_{27}H_{44}O_3$).

Description Calcitriol appears as white crystals.
Calcitriol is freely soluble in ethanol (95), soluble in fatty oils, practically insoluble in water.
Calcitriol is sensitive to air, heat and light.

A reversible isomerisation to pre-calcitriol takes place in solution, depending on temperature and time.

Identification (1) Determine the infrared spectra of Calcitriol and Calcitriol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Retention time of the main peak obtained from test solution and standard solution in the Assay is the same.

Purity ***Related substances***—Weigh accurately 10 mg of Calcitriol, add acetonitrile, dissolve without heating, add acetonitrile to make 55 mL, add 2-amino-2-hydroxymethyl-1,3-propandiol buffer solution to make 100 mL, and use this solution as the test solution. Separately, weigh accurately 10 mg of Calcitriol RS, add acetonitrile, dissolve without heating, add acetonitrile to make 55 mL, add 2-amino-2-hydroxymethyl-1,3-propandiol buffer solution to make 100 mL, and use this solution as the standard solution. Perform the test with 50 μL of the test solution as directed under Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the peaks other than pre-calcitriol and calcitriol from the test solution by the area percentage method: triazoline adduct of pre-calcitriol having the relative retention time of 0.43 is not more than 0.1 %, *trans*-calcitriol {(5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,25-triol} having the relative retention time of 0.96 is not more than 0.25 %, 1β-calcitriol {(5*Z*,7*E*)-9,10- secocholesta -5,7,10(19)- triene -1β,3β,25--triol} having the relative retention time of 1.15 is not more than 0.1 %, methylene calcitriol {(5*Z*,7*E*)-1α,3β-dihydroxy-17-((*R*)-7-hydroxy-7-methyloctan-2-yl)-9,10-secoandrosta-5,7,10(19)-triene} having the relative retention time of 1.5 is not more than 0.25 %, each unidentified related substance is not more than 0.1 %, and the total area of related substances is not more than 1.0 %. Exclude any peak with an area less than 0.1 %.

2-Amino-2-hydroxymethyl-1,3-propandiol buffer solution—Dissolve 1.0 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 900 mL of water, adjust the pH to 7.0 to 7.5 with phosphoric acid, and add water to make 1000 mL.

Operating conditions

Proceed as directed in the operating conditions in the Assay under Calcitriol.

System suitability

System performance: When the procedure is run with 50 μL of the system suitability solution under the above operating conditions, the relative retention time of pre-calcitriol with respect to calcitriol is 0.9 with the resolution between these peaks being not less than 3.5. When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates is not less than 10000.

System repeatability: When the test is repeated 5 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation is not more than 1.0 %.

Time span of measurement: About 2 times as long as the retention time of calcitriol

System suitability solution—Warm 2 mL of the standard solution at 80 °C for 30 minutes.

Assay Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air. Weigh accurately 1 mg of Calcitriol, and dissolve in the mobile phase to make exactly 10 mL, use this solution as the test solution. Weigh accurately 1 mg of Calcitriol RS, and dissolve in the mobile phase to make exactly 10 mL, and use this solution as the standard solution (1). Add the mobile phase to 1.0 mL of this solution to make exactly 100 mL, use this solution as the standard solution (2). Keep 2 mL of standard solution (1) at 80 ºC for 30 minutes, use this solution as the standard solution (3). Perform the test with 50 μL each of test solution and standard solution (1) as directed under Liquid Chromatography according to the following conditions, and calculate the peak areas of Calcitriol of each solution, A_T and A_S, respectively.

$$\text{Amount (mg) of calcitriol } (C_{27}H_{44}O_3) = 10 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Calcitriol obtained from standard solution (1).

Operating conditions

Detector: Ultraviolet-visible absorption Photometer (wavelength: 230 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for the liquid chromatography.

Column temperature: A constant teperature of about 40 ºC.

Mobile phase: A mixture of acetonitrile and 2-amino-2-hydroxymethyl-1,3-propandiol buffer solution (550 : 450).

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution (3) under the above operating conditions: the relative retention time of pre-calcitriol peak to calcitriol peak is about 0.9, the resolution between the peaks due to Calcitriol and pre-calcitriol is not less than 3.5. Perform the test with 50 μL of the standard solution (1) according to the above operating conditions, the number of theoretical plates is not than 10000.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution (1) un-

der the above operating conditions: the relative standard deviation of peak area of calcitriol is not more than 1 %.

2-Amino-2-hydroxymethyl-1,3-propandiol buffer solution—Dissolve 1.0 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 1000 mL of water. Adjust the pH between 7.0 and 7.5.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and at a temperature between 2 and 8 ºC. The contents of an opened container are to be used immediately.

Calcium Chloride Hydrate

$CaCl_2 \cdot 2H_2O$: 147.02

Calcium Chloride Hydrate contains not less than 96.7 % and not more than 103.3 % of calcium chloride hydrate ($CaCl_2 \cdot 2H_2O$).

Description Calcium Chloride Hydrate is a white granule or mass and is odorless.
Calcium Chloride Hydrate is very soluble in water, soluble in ethanol, and practically insoluble in ether.
Calcium Chloride Hydrate is deliquescent.

Identification A solution of Calcium Chloride Hydrate (1 in 10) responds to the Qualitative Test for calcium salt and for chloride.

pH Dissolve 1.0 g of Calcium Chloride Hydrate in 20 mL of freshly boiled and cooled water: the pH of this solution is between 4.5 and 9.2.

Purity (1) ***Clarity and color of solution***—A solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of water is clear and colorless.

(2) ***Hypochlorite***—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 to 3 drops of dilute hydrochloric acid and 2 to 3 drops of zinc iodide-starch TS: no blue color is observed immediately.

(3) ***Sulfate***—Take 1.0 g of Calcium Chloride Hydrate and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Calcium Chloride Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Magnesium and alkali metals***—Dissolve 1 g of Calcium Chloride Hydrate in 50 mL of water, add 0.5 g of ammonium chloride, and heat for 1 minute. Immediately add 40 mL of oxalic acid TS, stir well until precipitation is well established, and add 2 drops of methyl red TS to the warm mixture. Add ammonia TS dropwise until the mixture is alkaline, cool to room temperature, add water to make 100 mL, mix, and allow to stand for 4 hours to overnight. Filter this solution, put 50 mL of the clear filtrate in a platinum dish, add 0.5 mL of sulfuric acid, evaporate to dryness in a steam bath, then heat gradually to complete volatization of ammonium salts, and ignite the residue to constant mass: not more than 5 mg (not more than 1.0 %).

(6) ***Barium***—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 drops of dilute hydrochloric acid and 2 mL of potassium sulfate TS and allow to stand for 10 minutes: no turbidity is produced.

(7) ***Iron, aluminum or phosphate***—Dissolve, in a Nessler tube, 1.0 g of Calcium Chloride Hydrate in 20 mL of water and 1 drop of dilute hydrochloric acid, boil, then cool, add 3 drops of ammonia TS and heat the solution to boil: no turbidity or precipitate is produced.

(8) ***Arsenic***—Prepare the test solution with 1.0 g of Calcium Chloride Hydrate according to Method 1 and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.4 g of Calcium Chloride Hydrate and dissolve in water to make exactly 200 mL. Measure exactly 20 mL of this solution, add 40 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator and titrate with 0.02 mol/L of disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.02 mol/L
disodium ethylenediaminetetraacetate VS
= 2.9402 mg of $CaCl_2 \cdot 2H_2O$

Containers and Storage ***Containers***—Tight containers.

Calcium Chloride Injection

Calcium Chloride Injection is an aqueous solution for injection. Calcium Chloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of calcium chloride ($CaCl_2$: 110.98).
The concentration of Calcium Chloride Injection is expressed as the quantity of calcium chloride ($CaCl_2$: 110.98).

Method of Preparation Prepare as directed under Injections, with Calcium Chloride Hydrate.

Description Calcium Chloride Injection is a clear colorless liquid.

Identification Calcium Chloride Injection responds to the Qualitative Test for calcium salt and for chloride.

pH 4.5 ~ 7.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.30 EU/mg of Calcium Chloride Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

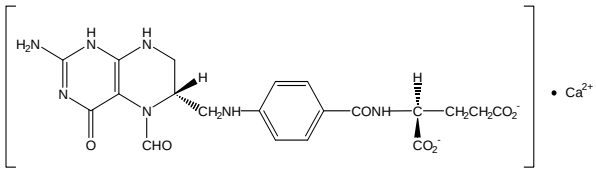
Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Calcium Chloride Injection, equivalent to about 0.4 g of calcium chloride ($CaCl_2$) and proceed as directed in the Assay under Calcium Chloride Hydrate.

Each mL of 0.02 mol/L
disodium ethylenediaminetetraacetate VS
= 2.2197 mg of $CaCl_2$

Containers and Storage ***Containers***—Hermetic containers.

Calcium Folinate



Calcium Leucovorin $C_{20}H_{21}CaN_7O_7$: 511.50

Calcium(2*S*)-2-{[4-[(*R*)-2-amino-5-formyl-4-oxo-5,6,7,8-tetrahydro-1*H*-pteridin-6-yl)methylamino]benzoyl]amino}pentanedioate [*1492-18-8*]

Calcium Folinate contains not less than 95.0 % and not more than 102.0 % of calcium folinate ($C_{20}H_{21}CaN_7O_7$), calculated on the anhydrous basis.

Description Calcium Folinate is a white to pale yellow powder, is odorless and tasteless.
Calcium Folinate is very soluble in water, freely soluble in acetic acid (100) and practically insoluble in ethanol (95) or in ether.
Calcium Folinate is gradually affected by light.

Identification (1) Determine the absorption spectra of solutions (1 in 100000) of Calcium Folinate and Calcium Folinate RS as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Calcium Folinate and Calcium Folinate RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Calcium Folinate (1 in 100) responds to the Qualitative Tests (2) and (3) for calcium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +14 ~ +19° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40 °C, if necessary, to dissolve: the pH of this solution is between 6.8 and 8.0.

Purity (1) ***Clarity of solution***—To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40 °C, if necessary, to dissolve: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry is not more than 0.25.

(2) ***Heavy metals***—Proceed with 0.40 g of Calcium Folinate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 50 ppm).

(3) ***Related substances***—Weigh accurately 10 mg of Calcium Folinate, dissolve in exactly 25 mL of water, and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the area percentage method: the area of any peak other than folinate from the test solution is not larger than the peak area of folinate from the standard solution, and the total area of the peaks other than folinate from the test solution is not larger than 5 times the peak area of folinate from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of the standard solution, and dissolve in water to make 50 mL. Confirm that the peak area of folinate obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard

deviation of the peak area of folinate is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of folinate, beginning after the solvent peak.

Water 7.0 ~ 17.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 10 mg each of Calcium Folinate and Calcium Folinate RS (previously determine the water), dissolve each in water to make exactly 25 mL, pipet 5 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of folinate in each solution.

$$\text{Amount (mg) of calcium folinate } (C_{20}H_{21}CaN_7O_7) = \text{Amount (mg) of Calcium Folinate RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C

Mobile phase: Adjust the pH of a mixture of disodium hydrogen phosphate dodecahydrate solution (287 in 100000), methanol, and tetrabutylammonium hydroxide TS (385 : 110 : 4) to 7.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of folinate is about 10 minutes.

System suitability

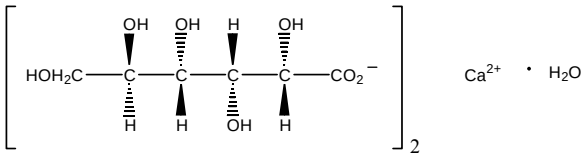
System performance: Dissolve 10 mg each of Calcium Folinate and folic acid in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, calcium folinate and folic acid are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of each peak area of folinate is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Calcium Gluconate Hydrate



Calcium Gluconate $C_{12}H_{22}CaO_{14}\cdot H_2O$: 448.39

Calcium (2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxy - hexanoate, hydrate [*299-28-5*]

Calcium Gluconate Hydrate, when dried, contains not less than 99.0 % and not more than 104.0 % of calcium gluconate hydrate ($C_{12}H_{22}CaO_{14}\cdot H_2O$).

Description Calcium Gluconate Hydrate is a white, crystalline powder or granule.

Calcium Gluconate Hydrate is freely soluble in hot water, soluble in water and practically insoluble in ethanol (99.5).

Identification (1) To 10 mg each of Calcium Gluconate Hydrate and Calcium Gluconate Hydrate RS, add 1 mL of water each, dissolve by warming, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), water, ammonia solution and ethyl acetate (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and heat the plate at 110 °C for 20 minutes. After cooling, spray evenly a mixture containing molybdate sulfate TS and cerium sulfate TS on the plate, air-dry, and heat at 110 °C for 10 minutes: the spots with the test solution and the standard solution are the same in the R_f value and color tone.

(2) A solution of Calcium Gluconate Hydrate (1 in 40) responds to the Qualitative Tests for calcium salt.

Specific Optical Rotation n_D^{20} : +6 ~ +11° (after drying, 0.5g, water, 25 mL, warm, after cooling, 100 mm).

pH Dissolve 1.0 g of Calcium Gluconate Hydrate in 20 mL of water by warming: the pH of the solution is between 6.0 and 8.0.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Calcium Gluconate Hydrate in 50 mL of water by warming: the solution is clear.

(2) ***Chloride***—Take 0.40 g of Calcium Gluconate Hydrate and perform the test. Prepare the control solution with 0.80 mL of 0.010 mol/L hydrochloric acid VS (not more than 0.071 %).

(3) ***Sulfate***—Take 1.0 g of Calcium Gluconate Hy-

drate and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) ***Phosphate***—To 10.0 g of Calcium Gluconate Hydrate, add 90 mL of about 70 °C to 80 °C and boil for 10 seconds until the solution becomes clear. Pipet 1 mL of this solution, add water to make 100 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of monopotassium phosphate and dissolve in water to make a solution containing 0.716 mg per mL. Pipet 1.0 mL of this solution and add water to make 100 mL. Pipet 2.0 mL of this solution, add water to make 100 mL and use this solution as the standard solution. To the test solution and the standard solution, add 4 mL of sulfomolybdic TS and 0.1 mL of a mixture of 3 mol/L hydrochloric acid TS and acidic tin (II) chloride TS (10:1) and combine. Allow to stand for 10 minutes: the color obtained from the test solution is not more intense than the color obtained from the standard solution (not more than 0.01 %).

(5) ***Heavy metals***—Dissolve 1.0 g of Calcium Gluconate Hydrate in 30 mL of water and 2 mL of dilute acetic acid by warming, cool and add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) ***Iron***—Place 1.0 g of Calcium Gluconate Hydrate in a 100 mL quartz glass flask, add 20 mL of 12 mol/L nitric acid TS and heat until fumes evolve. Add 0.5 mL of 30% hydrogen peroxide and heat again until fumes evolve. Repeat this procedure until the volume is reduced to about 5 mL and allow to cool. Add 1.0 mL of perchloric acid and boil. Do not heat to 190 °C or higher or evaporate to dryness as there is a risk of explosion. To this solution, add 2 mol/L hydrochloric acid TS to make 25 mL and use this solution as the test solution. Separately, pipet 2.0 mL, 4.0 mL and 10.0 mL of standard iron solution and transfer each to a 100 mL volumetric flask. Add 1.37 g of calcium chloride dehydrate and dilute with 2 mol/L hydrochloric acid TS to make 100 mL and use these solutions as standard solutions (1), (2) and (3), respectively. Separately, prepare 0.34 g of calcium chloride dehydrate in the same manner as the test solution and use this solution as the blank. Perform the test with the test solution and the standard solutions (1), (2) and (3) as directed under the Atomic Absorption Spectrophotometry according to the following conditions and use the calibration curves obtained from the absorbances of the standard solutions to determine the iron content of the test solution: not more than 5 ppm.

Gas used: Dissolved acetylene – Air
Lamp: Iron hollow-cathode lamp
Wavelength: 248.3 nm

(7) ***Magnesium and alkali metals***—Completely dissolve 1.0 g of Calcium Gluconate Hydrate in 100 mL of boiling water and add 10 mL of ammonium chloride TS, 1 mL of ammonia solution (28) and 50 mL of ammonium oxalate of 70 °C to 80 °C. Allow to stand for 4 hours then add water to make 200 mL and filter. Evaporate 100 mL of the filtrate to dryness and ignite to a constant mass: the residue is not more than 2 mg (not more than 0.4 %).

(8) ***Arsenic***—Dissolve 0.6 g of Calcium Gluconate Hydrate in 5 mL of water by warming, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS and concentrate in a water-bath to 5 mL use this solution as the test solution and perform the test (not more than 3.3 ppm).

(8) ***Sucrose and reducing sugars***—To 0.5 g of Calcium Gluconate Hydrate, add 10 mL of water and 2 mL of dilute hydrochloric acid and boil the solution for 2 minutes. After cooling, add 5 mL of sodium carbonate TS, allow to stand for 5 minutes, add water to make 20 mL and filter. To 5 mL of the filtrate, add 2 mL of Fehling's TS and boil for 1 minute: no orange to red precipitate is produced immediately.

Loss on Drying Not more than 1.0 % (1 g, 80 ºC, 2 hours).

Assay Weigh accurately 0.4 g of Calcium Gluconate Hydrate, previously dried, dissolve in 100 mL of water, add 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator and titrate immediately with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 22.420 mg of $C_{12}H_{22}CaO_{14}\cdot H_2O$

Containers and Storage ***Containers***—Well-closed containers.

Calcium Gluconate Injection

Calcium Gluconate Injection is an aqueous solution for injection. Calcium Gluconate Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of total calcium (Ca: 40.08).
An adequate amount of calcium saccharate or other suitable calcium salts may be added as stabilizers.

Method of Preparation Prepare as directed under Injections, with Calcium Gluconate Hydrate. Indicate the amount of total calcium if any stabilizers are added.

Description Calcium Gluconate Injection is a clear and colorless liquid.

Identification (1) Pipet about 5.0 mL of Calcium Gluconate Injection. Add 0.7 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydrazine and heat

the solution in a water-bath for 30 minutes. Cool and perform the test according to Identification (1) under Calcium Gluconate Hydrate.

(2) An aqueous solution of Calcium Gluconate Injection (1 in 5) responds to the Qualitative Tests for calcium.

pH 6.0 ~ 8.2.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.17 EU/g of Calcium Gluconate Hydrate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

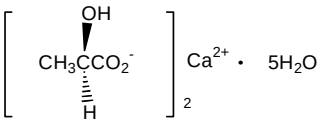
Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure accurately a volume of Calcium Gluconate Injection equivalent to about 0.4 g of Calcium Gluconate, add 100 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 mg of NN indicator. Immediately after the addition, titrate with 0.05 mol/L disodium ethylenediamine-tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium
ethlendiaminetetraacetate VS = 2.0040 mg of Ca

Containers and Storage ***Containers***—Hermetic containers.

Calcium Lactate Hydrate



$C_6H_{10}CaO_6 \cdot 5H_2O$: 308.29

Calcium (*RS*)-2-hydroxypropanoate pentahydrate
[*5743-47-5*]

Calcium Lactate Hydrate, when dried, contains not less than 97.0 % and not more than 101.0 % of calcium lactate ($C_6H_{10}CaO_6$: 218.22).

Description Calcium Lactate Hydrate is a white powder or granule, is odorless and has a slightly acid taste.
1 g Of Calcium Lactate Hydrate dissolves gradually in 20 mL of water, is slightly soluble in ethanol (95) and practically insoluble in ether.

Calcium Lactate Hydrate is partly efflorescent at ordinary temperature and yields the anhydride at 120 ºC.

Identification A solution of Calcium Lactate Hydrate (1 in 20) responds to the Qualitative Tests for calcium salt and for lactate.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Calcium Lactate Hydrate in 20 mL of water by warming: the solution is clear.

(2) ***Acid or alkali***—To the solution obtained in (1), add 2 drops of phenolphthalein TS: no red color is produced. Then add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) ***Heavy metals***—Dissolve 1.0 g of Calcium Lactate Hydrate in 30 mL of water and 5 mL of dilute acetic acid by warming, cool, add water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution and 2 mL of dilute acetic acid and dilute with water to make 50 mL (not more than 20 ppm).

(4) ***Magnesium or alkali metals***—Dissolve 1.0 g of Calcium Lactate Hydrate in 40 mL of water, add 0.5 g of ammonium chloride, boil, then add 20 mL of ammonium oxalate TS. Heat the mixture in a water-bath for 1 hour, cool, dilute with water to make 100 mL and filter, To 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness and ignite between 450 ºC and 550 ºC to a constant mass: the weight of the residue is not more than 5 mg.

(5) ***Arsenic***—Dissolve 0.5 g of Calcium Lactate Hydrate in 2 mL of water and 3 mL of hydrochloric acid, use this solution as the test solution. and perform the test (not more than 4 ppm).

(6) ***Volatile fatty acid***—Warm 1.0 g of Calcium Lactate Hydrate with 2 mL of sulfuric acid: an odor of acetic acid or butyric acid is not perceptible.

Loss on Drying 25.0 ~ 30.0 % (1 g, 80 ºC, 1 hour at first, then 120 ºC, 4 hours).

Assay Weigh accurately 0.5 g of Calcium Lactate Hydrate, previously dried, add water, dissolve by heating in a water-bath, cool and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS and allow to stand for 3 to 5 minutes. Add 0.1 g of NN indicator and titrate immediately with 0.02 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red to blue.

Each mL of 0.02 mol/L
disodium ethylenediaminetetraacetate VS
= 4.3644 mg of $C_6H_{10}CaO_6$.

Containers and Storage ***Containers***—Tight containers.

Calcium *p*-Aminosalicylate Granules

Pas-calcium Granules

Calcium *p*-aminosalicylate Granules contain not less than 95.0 % and not more than 105.0 % of the labeled amount of calcium *p*-aminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3½H_2O$: 254.25).

Method of Preparation Prepare as directed under Granules, with Calcium *p*-aminosalicylate Hydrate.

Identification Powder Calcium *p*-aminosalicylate Granules, weigh a portion of the powder, equivalent to 50 mg of calcium *p*-aminosalicylate Hydrate according to the labeled amount, add 100 mL of water, mix well and filter. To 10 mL of the filtrate, add 1 mL of 1 mol/L hydrochloric acid TS, shake and add 1 drop of iron (III) chloride TS: a red-purple color develops.

Dissolution Test Perform the test with an accurately weighed amount of Calcium *p*-aminosalicylate Granules, equivalent to about 250 mg of calcium *p*-aminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3½H_2O$) according to the labeled amount, at 75 revolutions per minute according to Method 2, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 60 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Calcium *p*-aminosalicylate Hydrate RS (previously determine the water in the same manner as Calcium *p*-aminosalicylate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at about 300 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Calcium *p*-aminosalicylate Granules in 60 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of calcium *p*-aminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3½H_2O$)

$$= \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 900 \times 1.330$$

W_S: Amount (g) of calcium *p*-aminosalicylate ($C_7H_5CaNO_3$) from Calcium *p*-aminosalicylate Hydrate RS, calculated on the anhydrous basis

W_T: Amount (g) of Calcium *p*-aminosalicylate Granules taken

C: Labeled amount (mg) of calcium *p*-aminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3½H_2O$) in 1 g

Particle Size Distribution Test for Preparations It meets the requirement.

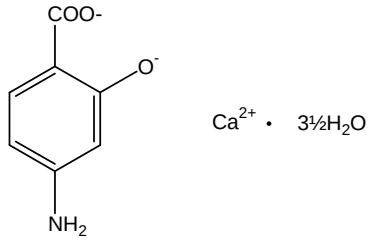
Assay Powder Calcium *p*-aminosalicylate Granules, weigh accurately a portion of the powder, equivalent to about 0.4 g of calcium *p*-aminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3½H_2O$: 251.23), add 120 mL of water and 1.5 mL of dilute hydrochloric acid and dissolve by heating on a water-bath. After cooling, add water to make exactly 200 mL and filter. Pipet 30 mL of the filtrate, transfer to an iodine flask and proceed as directed in the Assay under Calcium *p*-aminosalicylate Hydrate.

Each mL of 0.05 mol/L bromine VS
= 4.238 mg of $C_7H_5CaNO_3 \cdot 3½H_2O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Calcium *p*-Aminosalicylate Hydrate



Pas-calcium $C_7H_5CaNO_3 \cdot 3½H_2O$: 251.23

Calcium 4-amino-2-hydroxybenzoate hydrate
[*133-15-3*, anhydride]

Calcium *p*-aminosalicylate Hydrate contains not less than 97.0 % and not more than 103.0 % of calcium *p*-aminosalicylic acid ($C_7H_5CaNO_3$: 191.20), calculated on the anhydrous basis.

Description Calcium *p*-aminosalicylate Hydrate is a white to slightly colored powder, and has a slightly bitter taste.

Calcium *p*-aminosalicylate Hydrate is very slightly soluble in water and practically insoluble in ethanol (95) or in methanol.

Calcium *p*-aminosalicylate Hydrate is gradually colored to brown by light.

Identification (1) To 50 mg of Calcium *p*-aminosalicylate Hydrate, add 100 mL of water, shake well and filter. To 10 mL of the filtrate, add 1 mL of 1

mol/L hydrochloric acid TS, shake and add 1 drop of iron (III) chloride TS: a red-purple color develops.
(2) Determine the infrared spectra of Calcium *p*-aminosalicylate Hydrate and calcium *p*-aminosalicylate Hydrate RS, as directed in the potassium bromide disk method under Infrared Spectro-photometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.
(3) To 3 g of Calcium *p*-aminosalicylate Hydrate, add 15 mL of ammonium chloride TS and 15 mL of water, heat on a water-bath for 10 minutes: the solution responds to the Qualitative Tests (1), (2) and (3) for calcium salt.

Purity (1) ***Chloride***—Dissolve 1.0 g of Calcium *p*-aminosalicylate Hydrate in 15 mL of dilute nitric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.025 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Calcium *p*-aminosalicylate Hydrate according to method 3 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Dissolve 0.40 g of Calcium *p*-aminosalicylate Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS by warming on a water-bath, use this solution as the test solution and perform the test (not more than 5 ppm).

(4) **3-*Aminophenol***—To 0.10 g of Calcium *p*-aminosalicylate Hydrate, add 5 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate TS, previously cooled in ice-water and dissolve by shaking vigorously. Add immediately 3 mL of ammonia-ammonium chloride buffer solution, pH 11.0, previously cooled in ice-water and shake. Add 2 mL of 4-amino-*N,N*-diethylaniline sulfate TS, shake, add 10.0 mL of cyclohexane and 4 mL of diluted potassium hexacyanoferrate (III) TS (1 in 10) and shake immediately for 20 seconds. Centrifuge this solution, wash the separated cyclohexane layer with two 5 mL volumes of diluted ammonia TS (1 in 14), add 1 g of anhydrous sodium sulfate, shake and allow to stand for 5 minutes: the clear cyclohexane layer has no more color than the following control solution.

Control solution—Dissolve 50 mg of 3-aminophenol in water and dilute with water to make exactly 500 mL. Measure exactly 20 mL of this solution and add water to make exactly 100 mL. Take 5.0 mL of this solution, add 3 mL of ammonia-ammonium chloride buffer solution, pH 11.0, previously cooled in ice-water and treat this solution in the same manner as the test solution.

Water 23.3 ~ 26.3 % (0.1 g, volumetric titration, direct ditration)

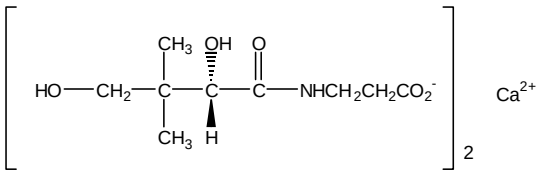
Assay Weigh accurately about 0.2 g of Calcium *p*-aminosalicylate Hydrate, dissolve in 60 mL of water and 0.75 mL of dilute hydrochloric acid by warming on a water-bath. After cooling, add water to make exactly 100 mL and use this solution as the test solution. Measure exactly 30 mL of the test solution, transfer to an iodine flask and add exactly 25 mL of 0.05 mol/L bromine VS and 20 mL of a solution of potassium bromide (1 in 4). Add immediately 14 mL of a mixture of acetic acid (100) and hydrochloric acid (5 : 2), stopper the flask immediately and allow to stand for 10 minutes with occasional shaking. Add cautiously 6 mL of potassium iodide TS and shake gently. After 5 minutes, titrate the produced iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary connection.

Each mL of 0.05 mol/L bromine VS
= 3.187 mg of $C_7H_5CaNO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Calcium Pantothenate



$C_{18}H_{32}CaN_2O_{10}$: 476.53

Calcium *bis*(3-{[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl]amino}propanoate) [*137-08-6*]

Calcium Pantothenate, when dried, contains not less than 5.7 % and not more than 6.0 % of nitrogen (N: 14.01) and not less than 8.2 % and not more than 8.6 % of calcium (Ca: 40.08).

Description Calcium Pantothenate is a white powder, is odorless and has bitter taste.
Calcium Pantothenate is freely soluble in water, very slightly soluble in ethanol (95) and practically insoluble in ether.

pH—The pH of a solution of Calcium Pantothenate (1 in 20) is between 7.0 and 9.0.
Calcium Pantothenate is hygroscopic.

Identification (1) Dissolve 50 mg of Calcium Pantothenate in 5 mL of sodium hydroxide TS and filter. Add 1 drop of copper (II) sulfate TS to the filtrate: a deep blue color develops.

(2) Add 5 mL of sodium hydroxide TS to 50 mg of Clacium Pantothenate, and boil for 1 minute. After cooling, add diluted hydrochloric acid (1 in 10) to adjust the solution to a pH between 3 and 4, and add 2

dops of iron (III) chloride TS: a yellow color develops.

(3) A solution of Calcium Panthothenate (1 in 10) responds to the Qualitative Tests for calcium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +25.0 ~ -28.5° (after drying, 1 g, water, 20 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Calcium Pantothenate in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Calcium Pantothenate according to method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Alkaloids***—Dissolve 50 mg of Calcium Pantothenate in 5 mL of water, add 0.5 mL of hexaammonium heptamolybdate TS and 0.5 mL of a solution of phosphoric acid (1 in 10): no white turbidity is produced.

(4) ***Related substances***—Dissolve 100 mg of Calcium Pantothenate in water to make exactly 10 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Beta Alanine RS in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the palte with a mixture of ethanol (95) and water (65 : 35) to a distance of about 15 cm, spray evenly a solution of ninhydrin in ethanol (95) (0.2 in 100), and air-dry the plate. Heat the plate at 120 °C for 20 minutes: any spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution (not more than 1.0 %).

Loss on Drying Not more than 5.0 % (1 g, 105 °C, 4 hours).

Assay (1) ***Nitrogen***—Proceed with about 50 mg of Calcium Pantothenate, previously dried and accurately weighed, as directed under Nitrogen Determination.

(2) ***Calcium***—Weigh accurately about 0.4 g of Calcium Pantothenate, previously dried, and dissolve in 30 mL of water by warming. After cooling, add exactly 25 mL of 0.05 mol/L disodium ethylenediamine tetraacetate VS, then 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and tittrate the excess disodium ethylenediamine tetraacetate with 0.05 mol/L magnesium chloride VS until the color of the solution changes from blue-purple to red-purple (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L disodium ethylenediamine tetraacetate VS = 2.0039 mg of Ca

Containers and Storage ***Containers***—Tight containers.

Calcium Polystyrene Sulfonate

Calcium 2-ethenylbenzenesulfonate [*37286-92-3*]

Calcium Polystyrene Sulfonate is a cation exchange resin prepared as the calcium form of the sulfonated styrene divinylbenzene copolymer.
Calcium Polystyrene Sulfonate, when dried, contains not less than 7.0 % and not more than 9.0 % of calcium (Ca: 40.08).
Each g of Calcium Polystyrene Sulfonate, when dried, exchanges with 53 to 71 mg of potassium (K: 39.10).

Description Calcium Polystyrene Sulfonate is a pale yellowish white to pale yellow powder, is odorless and tasteless.
Calcium Polystyrene Sulfonate is practically insoluble in water, in ethanol (95) or in ether.

Identification (1) Take Calcium Polystyrene Sulfonate and Calcium Polystyrene Sulfonate RS, previously dried and determine the infrared spectra as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Mix 0.5 g of Calcium Polystyrene Sulfonate with 10 mL of dilute hydrochloric acid, filter and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

Purity (1) ***Ammonium***—Place 1.0 g of Calcium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue (not more than 5 ppm).

(2) ***Heavy metals***—Proceed with 2.0 g of Calcium Polystyrene Sulfonate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Calcium Polystyrene Sulfonate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Styrene***—To 10.0 g of Calcium Polystyrene Sulfonate, add 10 mL of acetone, shake for 30 minutes, centrifuge and use the clear supernatant liquid as the test solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, dilute with acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions. Determine the peak heights, H_T and H_S, of styrene for the test solution and the standard solution: H_T is not larger than H_S.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column, about 3 mm in internal diameter and about 2 m in length, packed with siliceous earth for gas chromatography (149 to 177 μm in particle diameter), coated with polyethylene glycol 20M at the ratio of 15 %.

Column temperature: A constant temperature of about 90 ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of styrene is about 9 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of styrene are not less than 800 and between 0.8 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of each peak height of styreme is not more than 5.0 %.

(5) ***Sodium***—Pipet 20 mL of the 50 mL solution obtained in Assay (1), add 0.02 mol/L hydrochloric acid to make exactly 500 mL and use this solution as the test solution. Separately, weigh accurately 0.2542 g of sodium chloride, previously dried at 130 ºC for 2 hours and dissolve in 0.02 mol/L hydrochloric acid to make exactly 1000 mL. Pipet a suitable volume of this solution and dilute with 0.02 mol/L hydrochloric acid to make a solution containing 1 to 3 μg of sodium (Na: 22.99) per mL and use these solutions as the standard solutions. Perform the test with the test solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry according to the following conditions and determine the amount of sodium in the test solution using the calibration curve obtained from the standard solutions: the amount of sodium is not more than 1 %.

Gas used: Dissolved acetylene – Air

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

Loss on Drying Not more than 10.0 % (1 g, in vacuum, 80 ºC, 5 hours).

Microparticles (1) Apparatus: Use an apparatus as shown in the figure.

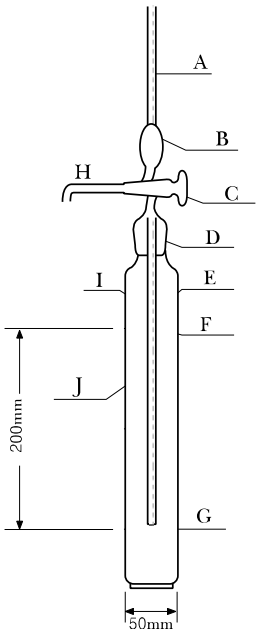
Actual volume to the mark of 20 cm at which the sedimentation tube is inserted: 550 mL.

Single suction volume: 10 mL.

(2) Procedure: Weigh accurately about 5.5 g of Calcium Polystyrene Sulfonate, previously dried, add 300 mL of water of 25 ºC and mix for 5 minutes. Transfer this turbid solution to the sedimentation tube, J, keeping a temperature at 25 ºC, add water of 25 ºC to 2 mm below the mark, F of 20 cm of the sedimentation tube, J and then insert the pipet. Open the two-way stopcock, C, exhaust air, add exactly water from the vent-hole, D to the mark, F of 20 cm and close the two-way stopcock, C. Shake the apparatus well vertically and horizontally, disperse Calcium Polystyrene Sulfonate in water and then open the two-way stopcock and allow to stand at 25 ± 1 ºC for 5 hours and 15 minutes. Then, draw exactly the meniscus of the turbid solution in sedimentation tube, J, up to the mark of pipet bulb, A, by suction, open the two-way stopcock, C, to the outlet of pipet, H and transfer exactly measured 20 mL of the turbid solution to a weighing bottle. Repeat the procedure and combine exactly measured 20 mL of the turbid solution. Evaporate 20 mL of this turbid solution on a water-bath to dryness, dry to constant mass at 105 ºC and weigh the residue as W_S (g). Pipet 20 mL of used water and weigh the residue in the same manner as W_B (g). Calculate the difference *mi* (g) of the weight between W_S and W_B and calculate the amount of microparticles (*S*) by the following equation: the amount of microparticles is not more than 0.1 %.

$$S\,(\%) = \frac{|W_S - W_B|\,(\mathrm{g}) \times V\,(\mathrm{mL})}{20\,(\mathrm{mL}) \times \text{amount of sample (g)}} \times 100$$

V: Actual volume (mL) to the mark of 20 cm at which the suction part of pipet is inserted.



A: Mark of pipet bulb
B: Pipet bulb for suction
C: Two-way stopcock
D: Vent-hole
E: Suction part of pipet
F: Mark of 20 cm
G: Base line of 0 cm
H: Outlet of pipet
I: Capillary tube of pipet
J: Sedimentation tube

Andreagen pipet

Assay (1) ***Calcium***—Weigh accurately about 1.0 g of Calcium Polystyrene Sulfonate, previously dried, and disperse in 5 mL of 3 mol/L hydrochloric acid TS. Transfer the mixture and wash out completely with the aid of a small volume of 3 mol/L hydrochloric acid TS, to a column, 12 mm in internal diameter and 70 mm in length, packed with a pledged of fine glass wool in the bottom, placing a volumetric flask as a receiver under the column. Then collect about 45 mL of eluate, adding

3 mol/L hydrochloric acid TS to the column and add water to make exactly 50 mL. Pipet 20 mL of this solution, adjust with ammonia water to a pH of exactly 10. Titrate immediately with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the red-purple color of the solution disappears and a blue color is observed (indicator: 40 mg eriochrome black T-sodium chloride indicator). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.0039 mg of Ca

(2) ***Potassium exchange capacity***—Pipet 50 mL of standard potassium stock solution into a glass-stoppered flask containing about 1.0 g of dried Calcium Polystyrene Sulfonate, accurately weighed, stir for 120 minutes, filter and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL and use this solution as the test solution. Separately, measure exactly a suitable volume of standard potassium stock solution, dilute with water to make solutions containing 0.5 to 2.5 μg of potassium (K: 39.10) per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions and determine the amount, Y (mg), of potassium in 1000 mL of the test solution, using the calibration curve obtained from the standard solution. The exchange capacity for potassium per g of dried Calcium Polystyrene Sulfonate is 53 to 71 mg, calculated by the following equation.

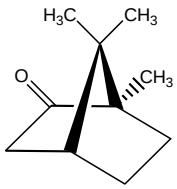
$$\text{Exchange capacity (mg) for potassium (K) per g of dried Calcium Polystyrene Sulfonate} = \frac{X - 100Y}{W}$$

X: Amount (mg) of potassium in 50 mL of standard potassium stock solution before exchange,
W: Amount (g) of dried Calcium Polystyrene Sulfonate taken.

Gas used: Dissolved acetylene – Air
Lamp: A potassium hollow-cathode lamp.
Wavelength: 766.5 nm.

Containers and Storage ***Containers***—Tight containers.

d-Camphor



$C_{10}H_{16}O$: 152.23

(1*R*,4*R*)-4,7,7-Trimethylbicyclo[2.2.1]heptan-3-one
[*464-49-3*]

d-Camphor contains not less than 96.0 % and not more than 101.0 % of *d*-camphor ($C_{10}H_{16}O$).

Description *d*-Camphor appears as colorless or white, translucent crystals, crystalline powder or mass. *d*-Camphor has a characteristic, agreeable odor and a slightly bitter taste, followed by a pleasant, cooling sensation.
Camphor is freely soluble in ethanol (95), in ether or in carbon disulfide and slightly soluble in water.
d-Camphor slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of d-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS and heat for 5 minutes in a water-bath: an orange-red precipitate is formed.

Specific Optical Rotation $[\alpha]_D^{20}$: +41.0 ~ +43.0° (5g, ethanol (95), 50 mL, 100 mm).

Melting Point 177 ~ 182 ºC

Purity (1) ***Water***—Shake 1.0 g of d-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) ***Chlorinated compounds***—Mix 0.20 g of finely powdered d-Camphor with 0.4 g of sodium peroxide in a dried, porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid and filter the solution into a Nessler tube. Wash the test tube and the filter with three 5 mL volumes of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution—Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) **Non-volatile residue**—Heat 2.0 g of d-camphor in a water-bath until sublimation is completed, then dry the residue at 105 ºC for 3 hours: the weight of the residue is not more than 1.0 mg.

Assay Weigh accurately 0.1 g each of *d*-Camphor and *d*-Camphor RS, add exactly 5 mL each of the internal standard solution, dissolve in ethanol (99.5) to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of *d*-Camphor to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of } d\text{-camphor } (C_{10}H_{16}O) = \text{Amount (mg) of } d\text{-Camphor RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl salicilate in ethanol (99.5) (1 in 25).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and 3 m in length, packed with 10 % of polyethylene glycol 20M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 160 ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of *d*-Camphor is about 6 minutes.

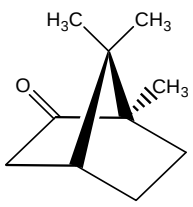
System suitability

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *d*-Camphor and the internal standard are eluted in this order, with a resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *d*-Camphor to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

dl-Camphor



and enantiomer

$C_{10}H_{16}O$: 152.23

4,7,7-Trimethylbicyclo[2.2.1]heptan-3-one [*76-22-2*]

dl-Camphor contains not less than 96.0 % and not more than 101.0 % of *dl*-camphor ($C_{10}H_{16}O$).

Description *dl*-Camphor appears as colorless or white, translucent crystals, crystalline powder or mass. *dl*-Camphor has a characteristic, agreeable odor and has a slightly bitter taste followed by a pleasant, cooling sensation.
dl-Camphor is freely soluble in ethanol (95), in ether or in carbon bisulfide and slightly soluble in water.
dl-Camphor slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of *dl*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenyl-hydradine TS and heat for 5 minutes in a water-bath: an orange-red precipitate is formed.

Specific Optical Rotation $[\alpha]_D^{20}$: -1.5 ~ +1.5° (5 g, ethanol (95), 50 mL, 100 mm).

Melting Point 175 ~ 180 ºC

Purity (1) ***Water***—Shake 1.0 g of dl-Camphor with 10 mL of carbon bisulfide: the solution is clear.

(2) ***Chlorinated compounds***—Mix 0.20 g of finely powdered dl-Camphor with 0.4 g of sodium peroxide in a dried, porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid and filter the solution into a Nessler tube. Wash the test tube and the filter with three 5 mL volumes of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution—Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) ***Non-volatile residue***—Heat 2.0 g of dl-Camphor in a water-bath until sublimation is completed, then dry the residue at 105 ºC for 3 hours: the weight of the residue is not more than 1.0 mg.

Assay Weigh accurately 0.1 g each of *dl*-Camphor and *dl*-Camphor RS, add exactly 5 mL each of the internal standard solution, dissolve in ethanol (99.5) to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of *dl*-camphor to that of the internal standard for the test

solution and the standard solution, respectively.

$$\text{Amount (mg) of } dl\text{-camphor } (C_{10}H_{16}O) = \text{Amount (mg) of } dl\text{-Camphor RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl salicilate in ethanol (99.5) (1 in 25).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and about 3 m in length, packed with 10 % of polyethylene glycol 20M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant tem-perature of about 160 ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of *dl*-Camphor is about 6 minutes.

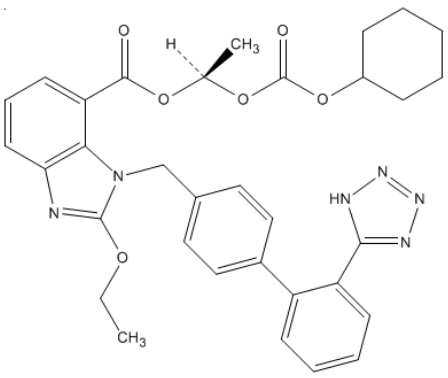
System suitability

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *dl*-Camphor and the internal standard are eluted in this order, with a resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *dl*-Camphor to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Candesartan Cilexetil



and enantiomer

$C_{33}H_{34}N_6O_6$: 610.66

1-Cyclohexyloxycarbonyloxyethyl 2-ethoxy-3-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]enzimidazole-4-carboxylate [*145040-37-5*]

Candesartan Cilexetil contains not less than 99.0 % and not more than 101.0 % of candesartan cilexetilm ($C_{33}H_{34}N_6O_6$), calculated on the anhydrous basis.

Description Candesartan Cilexetil appears as white crystals or crystalline powder.

Candesartan Cilexetil is soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Candesartan Cilexetil in methanol (1 in 100) shows no optical rotation.

Candesartan Cilexetil shows polylmorphism.

Identification (1) Determine the absorption spectra of solutions of Candesartan Cilexetil and Candesartan Cilexetil RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Candesartan Cilexetil and Candesartan Cilexetil RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Candesartan Cilexetil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 20 mg of Candesartan Cilexetil in 50 mL of a mixture of acetonitrile and water (3 : 2), and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of acetonitrile and water (3 : 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography, determine the peak areas of each solution by the automatic integration method, and calculate the amount of related substances: the area of the peaks, having the relative retention times of about 0.4 and about 2.0 with respect to candesartan cilexetil from the test solution, is not larger than 1/5 times the peak area of candesartan cilexetil from the standard solution, the area of the peak, having the relative retention time of about 0.5 with respect to candesartan cilexetil from the test solution, is not larger than 3/10 times the peak area of candesartan cilexetil from the standard solution, and the area of the peak other than candesartan cilexetil from the test solution and and the peaks mentioned above is not larger than 1/10 times the peak area of candesartan cilexetil from the standard solution. The total area of the peaks other than candesartan cilexetil from the test solution is not larger than 3/5 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of acetonitrile, water, and acetic acid (100) (57 : 43 : 1)

Mobile phase B: A mixture of acetonitrile, water, and acetic acid (100) (90 : 10 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-30	100→0	0→100

Flow rate: 0.8 mL/minute

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3 : 2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil obtained from 10 μL of this solution is equivalent to 7 to 13 % of that of candesartan cilexetil from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of candesartan cilexetil are not less than 12000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of candesartan cilexetil is not more than 2.0 %.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak

Water Not more than 0.3 % (0.5 g, coulometric titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Candesartan Cilexetil, dissolve in 60 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 61.07 mg of $C_{33}H_{34}N_6O_6$

Containers and Storage ***Containers***—Well-closed containers.

Candesartan Cilexetil Tablets

Candesartan Cilexetil Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$: 610.66).

Method of Preparation Prepare as directed under Tablets, with Candesartan Cilexetil.

Identification To an amount of powdered Candesartan Cilexetil Tablets, equivalent to 1 mg of candesartan cilexetil according to the labeled amount, add 50 mL of methanol, shake vigorously for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 252 and 256 nm and between 302 and 307 nm.

Purity ***Related substances***—Powder not less than 10 Candesartan Cilexetil Tablets, weigh accurately a portion of the powder, equivalent to 6 mg of candesartan cilexetil according to the labeled amount, add 15 mL of a mixture of acetonitrile and water (3 : 2), shake vigorously for 10 minutes, and centrifuge. Filter the clear supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the test solution. To 1 mL of this solution add a mixture of acetonitrile and water (3 : 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine the peak areas of each solution by the automatic integration method, and calculate the amount of related substances: the area of the peak, having the relative retention time of about 0.5 with respect to candesartan cilexetil from the test solution, is not larger than 1.5 times the peak area of candesartan cilexetil from the standard solution, the areas of the peaks, having the relative retention times of about 0.8, about 1.1, and about 1.5 with respect to candesartan cilexetil from the test solution, are not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, respectively, the area of the peak, having the relative retention time of about 2.0 with respect to candesartan cilexetil from the test solution, is not larger than the peak area of candesartan cilexetil form the standard solution, and the area of the peak other than the peak of candesartan cilexetil from the test solution, the peak having the relative retention time of about 0.4 with respect to candesartan cilexetil, and the peaks mentioned above is not larger than 1/10 times the peak area of candesartan cilexetil form the standard solution. The total area of the peaks other than candesartan cilexetil from the test solution is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of acetonitrile, water, and acetic acid (57 : 43 : 1)

Mobile phase B: A mixture of acetonitrile, water, and acetic acid (100) (90 : 10 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-30	100→0	0→100

Flow rate: 0.8 mL/minute

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3 : 2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil obtained from 10 μL of this solution is equivalent to 7 to 13 % of that of candesartan cilexetil from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of candesartan cilexetil are not less than 12000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of candesartan cilexetil is not more than 2.0 %.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak

Dissolution Test Perform the test with 1 tablet of Candesartan Cilexetil Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of a solution of polysorbate 20 (1 in 100) as the dissolution solution. Take not less than 20 mL of the dissolved solution 45 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL so that each mL contains about 2.2 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Candesartan Cilexetil RS (separately determine the water in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, and add acetonitrile to make 50 mL. Pipet 1 mL of this solution, add the dissolution solution to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of candesartan cilexetil in each solution. The dissolution rate of Candesartan Cilexetil Tablets in 45 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
= Amount (mg) of Candesartan Cilexetil RS, calculated on the anhydrous basis

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{18}{5}$$

C: Labeled amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in 1 tablet

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of candesartan cilexetil are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of candesartan cilexetil is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Candesartan Cilexetil Tablets. Weigh accurately a portion of the powder, equivalent to about 6 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), add exactly 15 mL of the internal standard solution, add a mixture of acetonitrile and water (3 : 2) to make 150 mL, shake vigorously for 10 minutes, and allow to stand. Filter the clear supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 50 mg of Candesartan Cilexetil RS (separately determine the water in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution and a mixture of acetonitrile and water (3 : 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the

following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
= Amount (mg) of Candesartan Cilexetil RS,
calculated on the anhydrous basis

$$\times \frac{Q_T}{Q_S} \times \frac{3}{25}$$

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 800)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of acetonitrile, water, and acetic acid (100) (57 : 43 : 1)

Flow rate: Adjust the flow rate so that the retention time of candesartan cilexetil is about 13 minutes.

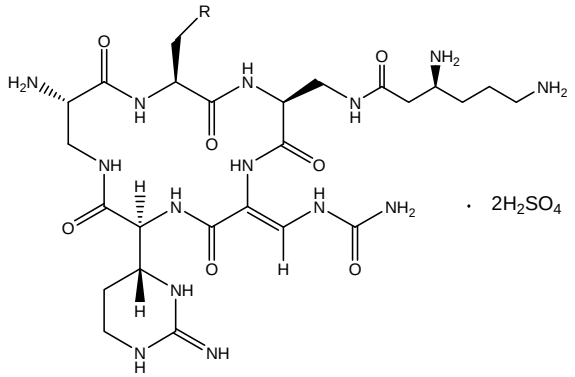
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditios, the internal standard and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of candesartan cilexetil to that of the internal stnadrd is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Capreomycin Sulfate



Capreomycin IA R = OH

Capreomycin IB R = H

Capreomycin IA
$C_{25}H_{44}N_{44}O_8 \cdot 2H_2SO_4$: 492.87
Capreomycin IB
$C_{25}H_{44}N_{44}O_7 \cdot 2H_2SO_4$: 484.87

3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)-iminohexahydro-pyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclo-hexadecan-5-yl}methyl)hexanamide [*1405-37-4*]

Capreomycin Sulfate contains not less than 700 μg (potency) per mg of capreomycin, calculated on the dried basis.

Description Capreomycin Sulfate appears as white to pale yellowish white crystalline powder or powder.
Capreomycin Sulfate is very soluble in water, and practically insoluble in ethanol (95), in chloroform, or in ether.

Identification (1) Dissolve a suitable amount of Capreomycin Sulfate in 0.1 mol/L hydrochloric acid TS to make a 0.002 % solution, and determine the absorption spectrum between 230 and 350 nm of this solution as directed under Ultraviolet-visible Spectrophotometry with a path length of 200 mm: it exhibits a maximum at around 268 nm.

(2) Dissolve a suitable amount of Capreomycin Sulfate in 0.1 mol/L sodium hydroxide TS to make a 0.002 % solution, and determine the absorption spectrum between 230 and 350 nm of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum at around 287 nm.

(3) Weigh about 5 mg (potency) each of Capreomycin Sulfate and Capreomycin Sulfate RS, transfer into test tubes, dissolve in 0.5 mL of hydrochloric acid and 0.5 mL of water, stopper tightly, warm at 100 °C for 16 hours, and evaporate to dryness in a water bath until the odor of hydrogen chloride gas is no longer perceptible. Dissolve each residue in 1 mL of water, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of microcrystalline cellulose for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethyl methyl ketone, and 1 mol/L hydrochloric acid TS (60 : 15 : 25). Remove the plate, dry in cold air for 15 minutes, warm at 100 °C for 15 minutes, and allow to cool. Spray evenly cadmium-ninihydrin TS on the plate and warm at 100 °C for 30 minutes: the spots from the test solution and standard solution have the same R_f value.

(4) A solution of Capreomycin Sulfate responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -26 ~ -36° (0.3 g, water, 30 mL, 200 mm).

pH The pH of a solution obtained by dissolving 3 g (potency) of Capreomycin Sulfate in 100 mL of water is between 4.5 and 7.5.

Purity ***Heavy metals***—Proceed with 1.0 g of Capreomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

Loss on Drying Not more than 10.0 % (0.1 g, 0.7 kPa, 100 °C, 4 hours).

Residue on Ignition Not more than 3.0 % (1 g, 700 °C).

Sterility Test It meets the requirement, when Capreomycin Sulfate is used in a sterile preparation.

Bacterial Endotoxinss Less than 0.35 EU/mg (potency) of capreomycin, when Capreomycin Sulfate is used in a sterile preparation.

Histamine It meets the requirement of the Histamine Test, when Capreomycin Sulfate is used in a sterile preparation. Dissolve a suitable amount of Capreomycin Sulfate in isotonic sodium chloride injection so that each mL contains 3.0 mg (potency), and use this solution as the test solution.

Capreomycin I Weigh accurately about 0.2 g of Capreomycin Sulfate, transfer to a 10 mL volumetric flask, and add water to make exactly 10 mL. Keep this test solution at a temperature not exceeding 5 °C. Perform the test with this solution as directed under Paper Chromatography. Use a glass chamber for the descending method measuring 300 × 300 × 600 mm as the developing chamber. Fill the glass chamber with a mixture of 1-propanol and water (7 : 3) to a height of 40 mm from the bottom, and saturate for 2 days. The migration rates of capreomycin I and II are influenced by this degree of saturation, so adjust the ratio of the mixture so that the R_f values of capreomycin I and II after developing are about 0.5 and about 0.6, respectively. Spot 100 μL of the test solution on a Whatman No. 1 filter paper for paper chromatography, 200 × 500 mm, or equivalent, and dry in warm air. Develop the filter paper with a mixture of 1-propanol, water, triethylamine, and acetic acid (100) (75 : 33 : 8 : 8) in the above developing chamber for 16 hours using the descending method, together with a separate filter paper for the blank test, and dry at room temperature for 1 hour. Examine the dried filter paper under ultraviolet light (main wavelength: 254 nm): capreomycin I and II appear at the R_f values of about 0.5 and about 0.6, respectively. Mark the principal spot of capreomycin, and mark the same position on the chromatogram of the blank test. Cut out this section, and cut again to make about 1.5 cm^2, respectively. Put into a 50 mL volumetric flask, add 0.1 mol/L acetate buffer solution (pH 6.2) to make exactly 50 mL, pipet 3.0 mL of this solution, transfer to a 50 mL volumetric flask, add water to make exactly 50 mL, and use this solution as the sample determination solution. Determine the absorbances at 268 nm of the above sample extract and sample determination solution with a path length of 10 mm, using water as the blank, and calculate the content of capreomycin I as follows (not less than 90.0 % with respect to the total amount of capreomycin). If the content of capreomycin I is not more than 90.0 %, perform the test two more times, perform a total capreomycin recovery test as follows, and use the average value of the three tests as the result.

$$\text{Content (\%) of capreomycin I} = \frac{A_I - A_b}{A_S} \times 100$$

A_I: Absorbance of the extract of the section of capreomycin I from the filter paper spotted with the sample.

A_b: Absorbance of the extract of the section corresponding to capreomycin I from the filter paper for the blank test.

A_S: Absorbance of the sample determination solution.

Total capreomycin recovery test: Spot 100 μL of the test solution on the origin line of a filter paper, dry in warm air, cut out the spotted section and the same section of a filter paper that has not been spotted, cut again to make 1.5 cm^2, and put into two 50 mL conical flasks. Proceed as directed above, and calculate the recovery rate of total capreomycin by the following equation (recovery rate of total capreomycin: 100 ± 2 %).

$$\text{Content (\%) of capreomycin I} = \frac{A_T - A_b}{A_S} \times 100$$

A_T: Absorbance of the sample extract

A_b: Absorbance of the blank extract

A_S: Absorbance of the sample determination solution

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (2) ② under Microbial Assay for Antibiotics.

(2) Test organism- *Micrococcus luteus* ATCC 9341.

(3) Dissolve a suitable amount of Capreomycin Sulfate in sterile purified water so that each mL contains 8 mg (potency). Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 800.0 μg (potency) and 200.0 μg (potency), and use these solutions as the high

concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 80 mg (potency) of Capreomycin Sulfate RS, dissolve in sterile purified water so that each mL contains 8 mg (potency), and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 800.0 μg (potency) and 200.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Capreomycin Sulfate for Injection

Capreomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.
Capreomycin Sulfate for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of capreomycin.

Method of Preparation Prepare as directed under Injections, with Capreomycin Sulfate.

Description Capreomycin Sulfate for Injection appears as white to pale yellowish white powder.

Identification Proceed as directed in the Identification (1), (2), and (3) under Capreomycin Sulfate.

pH The pH of a solution obtained by dissolving an amount of Capreomycin Sulfate for Injection, equivalent to 0.3 mg (potency) of capreomycin, in 10 mL of water is between 4.5 and 7.5.

Purity ***Heavy metals***—Proceed with 1.0 g of Capreomycin Sulfate for Injection according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

Loss on Drying Not more than 10.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 3.0 % (1.0 g). Moisten the carbonized residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.35 EU/mg (potency) of capreomycin.

Histamine It meets the requirement of the Histamine Test under Capreomycin Sulfate.

Foreign Insoluble Matter Test It meets the requirement.

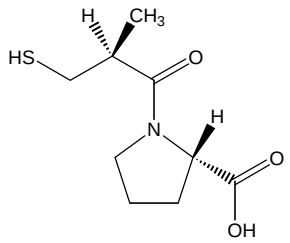
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Capreomycin Sulfate. Weigh accurately a suitable amount of Capreomycin Sulfate for Injection, dissolve in sterile purified water so that each mL contains 8 mg (potency), pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Captopril



$C_9H_{15}NO_3S$: 217.29

(2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid
[*62571-86-2*]

Captopril contains not less than 97.5 % and not more than 102.0 % of captopril ($C_9H_{15}NO_3S$), calculated on the dried basis.

Description Captopril appears as white crystals or crystalline powder.
Captopril is very soluble in methanol, freely soluble in ethanol (99.5) and soluble in water.
Melting points—About 106 ºC

Identification Determine the infrared spectra of Captopril and Captopril RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -125 ~ -134° (dried, 0.1 g, ethanol (99.5), 10 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Captopril according to the Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Captopril according to Method 1 and perform the test. (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50.0 mg of Captopril in methanol to make exactly 25 mL and use this solution as the test solution(use immediately after preparation). Separately, weigh accurately captopril disulfide RS, dissolve in methanol to make 10 μg per mL and use this solution as the standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions and calculate the peak areas, A_T and A_S, of captopril disulfide of these solutions: the amount of captoprile disulfide is not more than 1.0 % and the peak area of each related substance is not more than 40 % of the main peak in the chromatogram of the standard solution(not more than 0.2 %) and the sum of the related substance peak area is not more than the main peak area in the chromatogram of the standard solution(not more than 0.5 %).

$$\text{Amount (\%) of captopril disulfide} = \frac{C_S}{C_u} \times \frac{A_T}{A_S} \times 100$$

C_S: the concentration of captopril disulfide RS in the standard solution (μg/mL)

C_u: the concentration of captopril in the test solution (μg/mL)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particles diameter).

Mobile phase: A mixture of tetrahydrofuran methanol solution (9 in 100) and phosphoric acid solution (1 in 2000) (33 : 67).

Selection of column: Dissolve Captopril RS, Captopril Disulfide RS and 3-Acetylthio-2-Methylpropanoic Acid RS in methanol to make 10 μg per mL, respectively. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of captopril, 3-acetylthio-2-methylpropanoic acid and captopril disulfide in this order with the resolution between peaks of captopril and 3-acetylthio-2-methylpropanoic acid being not less than 3.0.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, 80 ºC, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.3 g of Captopril, dissolve in 100 mL of water, add 20 mL of dilute sulfuric acid and 1 g of potassium iodide and shake. Titrate with 1/60 mol/L potassium iodate VS (indicator: 2 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 1/60 mol/L potassium iodate
= 21.729 mg of $C_9H_{15}NO_3S$

Containers and Storage ***Containers***—Tight containers.

Captopril Tablets

Captopril Tablets contain not less than 90.0 % and not more than 110.0 % of labeled amount of captopril ($C_9H_{15}NO_3S$: 217.29).

Method of Preparation Prepare as directed under Tablets, with Captopril.

Identification Weigh accurately and powder a portion of Captopril Tablets, equivalent to 0.1 g of Captopril, put in the flask, add 25 mL of methanol, mix for 30 minutes, centrifuge, and use the clear supernatant liquid as the test solution. Separately, weigh a suitable amount of Captopril RS, dissolve in a suitable amount of methanol so as to contain 4 mg of Captopril per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin Layer Chromatograph. Spot 50 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plates with a mixture of toluene, acetic acid (100) and methanol (75 : 25 : 1) to a distance of about 15 cm and air-dry the plate. Spray with a freshly prepared mixture of ammonia solution (28) and a solution of 0.04 % 5,5'-dithio-bis-(2-nitrobenzoic acid) (1 : 6). The R_f value of the principal spot of the test solution is the same as that of standard solution.

Purity ***Captoprilndisulfide***—Use the test solution under the Assay. Separately, weigh accurately a suitable portion of Captopril Disulfide RS and dissolve in a suitable volume of mobile phase so as to contain 50 μg of captopril disufide per mL. Use this solution as the standard solution. Avoid the test solution and the standard solution with contact of air and use them within 8 hours of preparation. Perform the test with 20 μL each of the test solution and the standard solution as directed under the the Liquid Chromatography according to the following operating conditions. Determine peak areas, A_T and A_S, of captopril disulfide of the test solution and the standard solution, respectively: the amount of cap-

topril disulfide is not more than 3.0 %.

$$\text{Amount (\%) of captopril disulfide} = \frac{2500 \cdot C}{W} \times \frac{A_T}{A_S}$$

C: Concentration of the standard solution (mg/mL),
W: Amount of sample (mg).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column:A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilylated silica gel (contains 15 % of CH group) for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of methanol, water and phosphoric acid (450 : 550 : 0.5).

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution, as directed under the above operating conditions, Captopril and captopril disulfide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation between the peak areas of captopril is not more than 2.0 %.

Dissolution Test Perform the test with 1 tablet of Captopril Tablets at 50 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid. as a dissolution solution. Take 20 mL or more of the dissolved solution 20 minutes after starting the test, filter and use the filtrate as the test solution. Separately, weigh accurately a suitable amount of Captopril RS, dissolved in a suitable amount of water so as to contain same amount of Captopril per mL as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 212 nm as directed under Ultraviolet-visible Spectrophotometry : The dissolution rate (%) with respect to the labeled amount of Captopril Tablets in 20 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay Transfer not less than 20 Captopril Tablets to a suitable volumetric flask, add mobile phase to fill the flask to about half of its capacity and sonicate for 15 minutes. Dilute with mobile phase to volume, shake by mechanical means for 15 minutes and filter. Dilute quantitatively and stepwise if necessary, with mobile phase to obtain a solution having a concentration of about 1 mg of Captopril per mL. Separately, dissolve suitable quantities of Captopril RS and Captopril Disulfide RS in mobile phase to obtain a solution having known concentrations of about 1 mg per mL and 50 μg per mL, respectively. Use this solution as the standard solution. Avoid the test solution and the standard solution with contact of air and use them within 8 hours of prepration. Proceed with 20 μL each of the test solution and the standard solution according to the Liquid Chromatography under the following operating conditions. Determine peak areas, A_T and A_S, of Captopril of the test solution and the standard solution, respectively.

$$\text{Amount(mg) of Captopril } (C_9H_{15}NO_3S) \text{ in 1 mL of test solution} = C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Captopril in the standard solution,

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm length, packed with octadecylsilylated silica gel (contains 15 % of CH group) for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture solution of methanol, water and phosphoric acid (450 : 550 : 0.5).

Flow rate: 1.0 mL/minute.

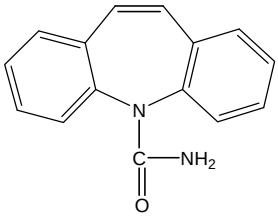
System suitability

System performance: When the procedure is run with 20 μL of the stndard solution, as directed under the above operating conditions, Captopril and captopril disulfide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak areas of Captopril is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Carbamazepine



$C_{15}H_{l2}N_2O$: 236.27

2-Azatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3(8),4,6,9,12,14-heptaene-2-carboxamide

[*298-46-4*]

Carbamazepine, when dried, contains not less than 97.0 % and not more than 103.0 % of carbamazepine ($C_{15}H_{12}N_2O$).

Description Carbamazepine is a white to slightly yellowish white powder, is odorless and tasteless at first and leaves a slightly bitter aftertaste.
Carbamazepine is freely soluble in chloroform, sparingly soluble in ethanol (95) or in acetone and very slightly soluble in water or in ether.

Identification (1) To 0.1 g of Carbamazepine, add 2 mL of nitric acid and heat in a water-bath for 3 minutes: an orange-red color is produced.

(2) To 0.1 g of Carbamazepine, add 2 mL of sulfuric acid and heat in a water-bath for 3 minutes: a yellow color is produced with a green fluorescence.

(3) Examine Carbamazepine under ultraviolet light: the solution shows an intense blue fluorescence.

(4) Determine the absorption spectra of solutions of Carbamazepin and Carbamazepin RS in ethanol (95) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 189 ~ 193 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Carbamazepine in 10 mL of chloroform: the solution is clear and colorless to pale yellow.

(2) ***Acid***—To 2.0 g of Carbamazepine, add exactly 40 mL of water, stir well for 15 minutes and filter through a glass filter. To 10 mL of this filtrate, add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(3) ***Alkali***—To 10 mL of the filtrate obtained in (2), add 1 drop of methyl red TS and 0.50 mL of 0.01 mol/L hydrochloric acid VS: a red color is produced.

(4) ***Chloride***—Dissolve 0.25 g of Carbamazepine in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.20 mL of 0.01 mol/L hydrochloric acid VS, add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028 %).

(5) ***Heavy metals***—Proceed with 2.0 g of Carbamazepine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Related substances***—Dissolve 0.25 g of Carbamazepine in 10 mL of chloroform and use this solution as the test solution. Separately, dissolve 5.0 mg of iminodibenzyl in chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and methanol (19 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

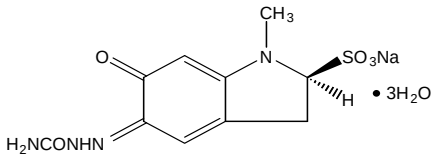
Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve 50 mg of Carbamazepine, previously dried and accurately weighed, in ethanol (95) to make exactly 250 mL. Dilute 5 mL of this solution with ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry and determine the absorbance, *A*, of this solution at the wavelength of a maximum absorption at about 285 nm.

$$\text{Amount (mg) of carbamazepine } (C_{15}H_{12}N_2O) = \frac{A}{490} \times 50000$$

Containers and Storage ***Containers***—Tight containers.

Carbazochrome Sodium Sulfonate Hydrate



and enantiomer

Carbazochrome Sodium Sulfonate
$C_{10}H_{11}N_4NaO_5S \cdot 3H_20$: 376.32

Sodium (5*Z*)-5-(carbamoylhydrazono)-1-methyl-6-oxo-2,3,5,6-tetrahydro-1*H*-indole-2-sulfonate hydrate

Carbazochrome Sodium Sulfonate Hydrate contains not less than 98.0 % and not more than 102.0 % of carbazochrome sodium sulfonate ($C_{10}H_{11}N_4NaO_5S$: 322.28), calculated on the anhydrous basis.

Description Carbazochrome Sodium Sulfonate appears as orange-yellow, crystals or crystalline powder.
Carbazochrome Sodium Sulfate is sparingly soluble in water, very slightly soluble in ethanol (95) and practically insoluble in ether.
A solution of Carbazochrome Sodium Sulfonate(1 in 100) shows no optical rotation.

Melting point —About 210 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Carbazochrome Sodium Sulfonate and Carbazochrome Sodium Sulfonate RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Carbazochrome Sodium Sulfonate and Carbazochrome Sodium Sulfonate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Carbazochrome Sodium Sulfonate (1 in 100) responds to the Qualitative Tests (1) for sodium salt.

pH Dissolve 0.8 g of Carbazochrome Sodium Sulfonate in 50 mL of water by warming and cool: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Carbazochrome Sodium Sulfonate in 50 mL of water by wanting and allow to cool: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 590 nm is not more than 0.070.

(2) ***Heavy metals***—Proceed with 1.0 g of Carbazochrome Sodium Sulfonate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 50 mg of Carbazochrome Sodium Sulfonate in 100 mL of water and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: the total area of the peaks other than the peak of carbazochrome sulfonate from the test solution is not larger than the peak area of carbazochrome sulfonate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 1.2 g of monobasic ammonium phosphate in 1000 mL of water and filter through a membrane filter, if necessary. To 925 mL of this solution, add 75 mL of ethanol (95), shake and adjust with phosphoric acid to a pH of 3.

Flow rate: Adjust the flow rate so that the retention time of carbazochrome sulfonate is between 6 and 8 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of carbazochrome sulfonate obtained from 10 μL of the standard solution composes about 5 % of the full scale.

Test for required detectability: Take exactly 2 mLof the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of Carbazochrome Sulfonate from 10 mL of this solution is 7 % to 13 % of the peak area of Carbazochrome Sulfonate from the standard solution.

System performance: Dissolve 10 mg each of Carbazochrome Sodium Sulfonate and carbazochrome in 100 mL of water by warming. When the procedure is run with 10 μL of this solution under the above operating conditions, Carbazochrome sulfonate and Carbazochrome are eluted in this order with the resolution between their peaks being not less than 3.

Time span of measurement: About 3 times as long as the retention time of carbazochrome sulfonate after the solvent peak.

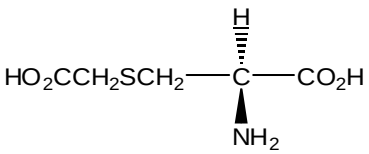
Water 13.0 ~ 16.0 % (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.25 g of Carbazochrome Sodium Sulfonate, dissolve in 50 mL of water, apply to a chromatographic column, 10 mm in diameter, previously prepared with 20 mL of strongly acidic ion exchange resin for column chromatography (type H) and allow to flow at a rate of 4 mL per minute. Wash the column with 150 mL of water, combine the washing and the former eluent solution and titrate with 0.05 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 16.114 mg of $C_{10}H_{11}N_4NaO_5S$

Containers and Storage ***Containers***—Well-closed containers.

L-Carbocysteine



Carbocysteine $C_5H_9NO_4S$: 179.19

S-(Carboxymethyl)-L-cysteine [*638-23-3*]

L-Carbocysteine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-carbocysteine ($C_5H_9NO_4S$).

Description L-Carbocysteine is a white crystalline powder, is odorless and has a slightly acid taste.
L-Carbocysteine is very slightly soluble in water and practically insoluble in ethanol (95), in glacial acetic acid or in ether.
L-Carbocysteine dissolves in dilute hydrochloric acid or in sodium hydroxide TS.

Melting point—About 186 ºC (with decomposition)

Identification (1) To 0.2 g of L-Carbocysteine, add 1 mL of lead acetate TS and 3 mL of water, shake, add 0.2 g of sodium hydroxide and heat over a flame for 1 minute: a dark brown to black precipitate is formed.

(2) Determine the infrared spectra of L-Carbocysteine and Carbocysteine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -33.5 ~ -36.5º. Weigh accurately 5 g of L-Carbocysteine, previously dried, dissolve in 20 mL of water and a suitable volume of a solution of sodium hydroxide (13 in 100) and adjust the pH with 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS to 6.0 and add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100 mm cell.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of L-Carbocysteine in 10 mL of a solution of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.20 g of L-Carbocysteine in 10 mL of water and 20 mL of nitric acid and add water to make 50 mL. Perform the test using this solution as the test solution.

Control solution—To 0.40 mL of 0.01 mol/L hydrochloric acid VS, add 20 mL of nitric acid and water to make 50 mL (not more than 0.071 %).

(3) ***Ammonium***—Perform the test with 0.25 g of L-Carbocysteine using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(4) ***Heavy metals***—To 2.0 g of L-Carbocysteine add 0.5 g of magnesium oxide, mix, and carbonize. After cooling, ignite to incinerate at a temperature not exceeding 800 °C for 1 hour. After cooling, dissolve the residue in 5 mL of a mixture of hydrochloric acid and water (1 : 1). Add 0.1 mL of phenolphthalein TS, and add ammonia solution (28) dropwise until the solution becomes pale red. After cooling, add acetic acid (100) until the color disappears, and add a further 0.5 mL. If necessary, filter and wash. Add water to make 20 mL, and use this solution as the test solution. Separately, proceed with 2.0 mL of standard lead solution instead of L-Carbocysteine in the same manner as the test solution. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the control solution. Separately, to 10 mL of water add 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution, add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

System suitability: The control solution shows a slightly brown color compared to the blank solution. To the test solution add 2.0 mL of standard lead solution. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the system suitability solution. The color of the system suitability solution is not less intense than that of the control solution.

(5) ***Arsenic***—Prepare the test solution with 1.0 g of L-Carbocysteine according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.30 g of L-Carbocysteine in 10 mL of 0.2 mol/L sodium hydroxide TS and use this solution as the test solution. Pipet 2 mL of the test solution and add 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 1 mL of this solution and add 0.2 mol/L sodium hydroxide TS to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution, in 15 mm length along the starting line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm, air-dry the plate and then dry at 80 ºC for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 ºC for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.25 g of L-Carbocysteine, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS and 50 mL of acetic acid (100) and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.919 mg of $C_5H_9NO_4S$

Containers and Storage ***Containers***—Tight containers.

Carbon Dioxide

CO_2: 44.01

[*124-38-9*]

Carbon Dioxide contains not less than 99.5 vol % and not more than 101.0 vol % of carbon dioxide (CO_2).

Description Carbon Dioxide is a colorless gas at room temperature and under atmospheric pressure and is odorless.
1 mL volume of Carbon Dioxide dissolve in 1 mL of water and the solution is slightly acidic.
1000 mL of Carbon Dioxide at 0 ºC and under a pressure of 101.3 kPa weighs about 1.978 g.

Identification (1) Put a flaming wood splinter into Carbon Dioxide: the flame is extinguished immediately.

(2) Pass Carbon Dioxide into calcium hydroxide TS: a white precipitate is produced. Collect the precipitate and add acetic acid: it dissolves with effervescence.

Purity Maintain containers of Carbon Dioxide between 18 ºC and 22 ºC for not less than 6 hours prior to the test and correct the volume of Carbon Dioxide to 20 ºC and under a pressure of 101.3 kPa.

(1) ***Acid***—Place 50 mL of freshly boiled and cooled water in a Nessler tube and pass 1000 mL of Carbon Dioxide for 15 minutes through an introducing tube about 1 mm in diameter extending to 2 mm from the bottom of the Nessler tube, then add 0.10 mL of methyl orange TS: the solution has no more color than the following control solution.

Control solution—To 50 mL of freshly boiled and cooled water in a Nessler tube, add 0.10 mL of methyl orange TS and 1.0 mL of 0.01 mol/L hydrochloric acid VS.

(2) ***Hydrogen phosphide, hydrogen sulfide or reducing organic substances***—Place 25 mL of silver nitrate-ammonia TS and 3 mL of ammonia TS in each two Nessler tubes A and B and designate the solution in each tube as solutions A and B, respectively. Pass 1000 mL of Carbon Dioxide into solution A in the same manner as directed in (1): the turbidity and color of this solution are the same as that of solution B.

(3) ***Carbon monoxide***—Introduce 5.0 mL of Carbon dioxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure reducing valve, through a directly connected polyvinyl tube. Perform the test with this according to the Gas Chromatography under the following conditions: no peak is observed at the same retention time as that of the carbon monoxide.

Operating conditions

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in internal diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in poreous size).

Column temperature: A constant temperature of about 50 ºC.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

System suitability

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the system performance is about 10 cm.

System performance: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL and mix well. When the procedure is run with 5.0 mL of the mixed gas under the above operating conditions, oxygen, nitrogen and carbon monoxide are eluted in this order with a well-resolving of their peaks.

(4) ***Oxygen and nitrogen***—Introduce 1.0 mL of Carbon Dioxide into a gas-measuring tube or syringe for gas chromatography from metal cylinder under pressure with a pressure-reducing valve through a directly connected polyvinyl chloride tube. Perform the test as directed under Gas Chromatography according to the following conditions and determine the peak area, A_T, of air. Separately, introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, allow to mix thoroughly and use this mixture as the standard gas mixture. Perform the test with 1.0 mL of the mixture in the same manner as directed in the case of Carbon Dioxide and determine the peak area, A_S, of nitrogen: A_T is smaller than A_S and no other peak appears.

Operating conditions

Detector: A thermal-conductivity detector.

Column: A column, about 3 mm in internal diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 μm in particle diameter).

Column temperature: A constant temperature of about 50 ºC.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of nitrogen obtained from 1.0 mL of the standard gas mixture composes about 50 % of the full scale.

System performance: Collect 0.5 mL of nitrogen in a gas mixer, add Carbon Dioxide to make 100 mL,

mix well and proceed with 1.0 mL of the mixture under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and Carbon Dioxide in this order.

(5) ***Nitric oxide***—Proceed in the same manner as directed in (6) Nitrogen dioxide. Pass 550 ± 50 mL of Carbon Dioxide in the gas phase through a nitric oxide-nitrogen dioxide detector tube at the specified rate, and determine the amount of nitric oxide: not more than 2.5 ppm.

(6) ***Nitrogen dioxide***—To a cylinder, connect a piece of tubing of sufficient length to allow all the contents in the liquid phase to vaporize during passage through it when the valve is opened, and prevent frost from reaching the inlet of the detector tube. Pass 550 ± 50 mL of Carbon Dioxide in the gas phase through a nitric oxide-nitrogen dioxide detector tube at the specified rate via the tubing (previously flush the system with Carbon Dioxide to displace air), and determine the amount of nitrogen dioxide: not more than 2.5 ppm. Measure the gases with a gas volume meter downstream from the detector tube in order to prevent contamination.

(7) ***Sulfur dioxide***—Proceed in the same manner as directed in (6) Nitrogen dioxide. Pass 1050 ± 50 mL of vaporized Carbon Dioxide through a sulfur dioxide detector tube at the specified rate, and determine the amount of sulfur dioxide: not more than 5 ppm.

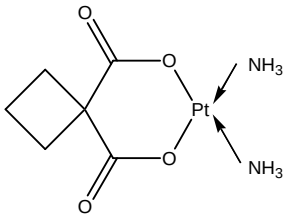
Assay For the withdrawing of Carbon Dioxide, proceed as directed in the Purity. Place 125 mL of a solution of potassium hydroxide (1 in 2) in a gas pipet of suitable capacity. Measure exactly about 100 mL of Carbon Dioxide in a 100 mL gas buret filled with water. Force the entire volume of gas into the gas pipet and shake for 5 minutes. Draw some of the unabsored gas into the gas buret, measure the volume, force the residual back upon the surface of the liquid in the gas pipet and repeat this procedure until a constant volume of the residual reading is obtained. Determine the volume, V (mL) of the residual gas and correct its volume, V to 20 ºC and under a pressure of 101.3 kPa.

Volume (mL) of carbon dioxide (CO_2)
= calculated volume (mL) of the sample
– calculated volume, V (mL)

Containers and Storage ***Containers***—Metal cylinders.

Storage—Not exceeding 40 °C.

Carboplatin



$C_6H_{12}N_2O_4Pt$: 371.25

cis-Diammine(cyclobutane-1,1-dicarboxylate-*O,O'*)platinum(II) [*41575-94-4*]

Carboplatin contains not less than 98.0 % and not more than 102.0 % of carboplatin ($C_6H_{12}N_2O_4Pt$), calculated on the anhydrous basis.

Description Carboplatin is a white crystalline powder.
Carboplatin is slightly soluble in water, and practically insoluble in acetone or in ethanol (95).

Melting point—About 200 ºC (with decomposition).

Identification Determine the infrared spectra of Carboplatin and Carboplatin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Crystallinity Test It meets the requirement.

pH pH of a solution of Carboplatin (1 in 100) is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of carboplatin in water to make exactly 10 mL and determine the transmittance of this solution using water as a blank as directed under Ultraviolet-visible Spectrophotometry at the 440 nm: not less than 97 %.

(2) ***Platinum***—Weigh accurately about 0.25 g of Carboplatin, add 400 mL of water, and slowly dissolve by heating almost to the boiling point, stirring frequently with a glass rod. Dissolve it complately, boil for about 10 minutes, cool for 1 minute without stirring, filter with quantitative filter paper. Transfer the filtrate to a 600-mL beaker, wash the quantitative filter paper with the warm water, combine the washings and the filterate. Evaporate this solution until to about 300 mL. Place a glass stirring rod in the beaker, and heat the solution to boiling, add slowly 10.0 mL of hydrazine hydrate to the center of beaker by dropwise addition. Add 2 drops of 10 mol/L sodium hydroxide TS, heat for about 10 minutes to coagulate the precipitate for ease of filtration, cool, filter with quantitative filter paper. Wash the beaker with warm water, filter the washing solution, wipe the beaker and stirring rod with a small piece of quantitative filter paper, place all the filter papers in the porcelain crucible, cover the cruci-

ble heat slowly to carbonize, then ignite at 800 ºC for 1 hour. Weigh the mass after cooling in the desicator with silica gel, determine the amount of the residue to the dehydrated carboplatin is between 52.0 and 53.0 %.

(3) ***Water-insoluble substances***—Weigh accurately about 1 g of Carboplatin, transfer to a beaker. Add 100 mL of water, and dissolve by stirring for 30 minutes. Filter through a tared glass filter. Rinse the beaker with water, and filter the rinsings. Dry the glass filter at 130 ± 10 ºC to constant mass: the amount of residue is not more than 0.5 %.

(4) ***1,1-cyclobutanedicarboxylic acid***—Weigh accurately about 50 mg of Carboplatin, dissolve in mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of 1,1-cyclobutanedicarboxylic acid, dissolve in mobile phase to make exactly 100 mL, add the mobile phase to 2.0 mL of this solution to make 200 mL, and use this solution as the standard solution. Perform the test with 100 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and measure the peak areas of 1,1-cyclobutanedicarboxylic acid obtained from the test solution and the standard solution, A_T and A_S, respectively, by the automatic integration method, calculate the amount of 1,1-cyclobutanedicarboxylic acid: not more than 0.5 %.

$$\text{Amount (\%) of 1,1-cyclobutanedicarboxylic acid} = 5 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C : Concentration (μg/mL) of 1,1-cyclobutane dicarboxylic acid in the standard solution.

W : Weight (mg) of Carboplatin taken to prepare the test solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength : 220 nm)

Column: A stainless steel column, about 4.0 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 mol/L sodium hydroxide to a pH of 7.55. Add 20 mL of this solution to a mixture of 880 mL of water and 100 mL of acetonitrile, mix.

Flow rate: 2 mL/minute

System suitability

System performance: Mix 1.0 mL of the standard solution and 1.0 mL of the standard solution in the Assay, and use this solution as the system suitability solution. When the procedure is run with 100 μL of this solution under the above operating conditions, the relative retention times of carboplatin peak and 1,1-cyclobutane dicarboxylic acid peak are 0.65 and 1.0, respectively, with the resolution between carboplatin and 1,1-cyclobutane dicarboxylic acid peaks being not less than 2.5, and the column efficiency, determined from the 1,1-cyclobutane dicarboxylic acid peak, is not less than 1500 theoretical plates.

System repeatability: when the test is repeated 6 times with 20 μL each of system suitability solution under the above operating conditions, the relative standard deviation of peak area obtained from 1,1-cyclobutane dicarboxylic acid is not more than 10 %.

(5) ***Related substances***—Dissolve 50 mg of Carboplatin in water to make exactly 50 mL, and use this solution as the test solution. Separately, dissolve 25 mg of Carboplatin RS in water to make exactly 100 mL, add water to 1.0 mL of this solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. The total peak areas other than carboplatin obtained from the test solution and 1,1-cyclobutanedicarboxylic acid by automatic integration method: is not more than 2 times peak area of carboplatin obtained from standard solution (0.5 %), and each peak area is not more than the peak area of carboplatin obtained from the standard solution (0.25 %).

Operating conditions

Detector, column, Mobile phase, System suitability correspond to the the operating conditions in the Assay.

Water Not more than 0.5 % (1 g, dehydraed form amide, volumetric titration, dirct titration).

Assay Weigh accurately about 50 mg each of Carboplatin and Carboplatin RS, dissolve in water to make exactly 50 mL, and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, measure the peak areas of Carboplatin and Carboplatin RS, A_T and A_S, by automatic integration method.

$$\text{Amount (mg) of carboplatin } (C_6H_{12}N_2O_4Pt) = \text{Amount (mg) of Carboplatin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column, about 4.0 mm in internal diameter and about 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (between 3 and 10 μm in particle diameter).

Mobile phase : A mixture of acetonitrile and water (87 : 13)

Flow rate: 2 mL/minute

System suitability

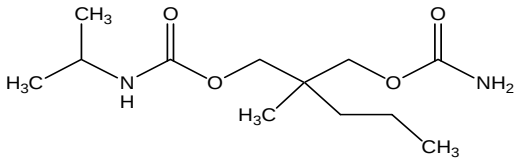
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the ratio of mass distribution is not less than 3.0, the numbers of theoretical plates is not less than 2500, and the symmetry factor of Carboplatin peak is not more than 2.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of peak area of carboplatin is not more than 1.2 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Carisoprodol



$C_{12}H_{24}N_2O_4$: 260.33

2-Methyl-2-({[(propan-2-yl)carbamoyl]oxy}methyl)pentyl carbamate [*78-44-4*]

Carisoprodol contains not less than 98.0 % and not more than 102.0 % of carisoprodol ($C_{12}H_{24}N_2O_4$), calculated on the dried basis.

Description Carisoprodol is a white, crystalline powder, has slightly specific odor and a bitter taste.

Carisoprodol is freely soluble in ethanol (95), in acetone or in chloroform and very slightly soluble in water.

Identification (1) Determine the infrared spectra of Carisoprodol and Carisoprodol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit slmilar intensities of absorption at the same wavenumbers.

(2) Weigh 0.1 g each of Carisoprodol and Carisoprodol RS, dissolve in 1.0 mL each of chloroform, and use these solutions as the test solution and the standard solution. Perform the test with these solutions according to meprobamate under the Purity: the main spots from the test solution and the standard solution have the same R_f value.

Melting Point 91 ~ 94 ºC.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Carisoprodol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Meprobamate***— Weigh 0.1 g of Carisoprodol and dissolve in 1.0 mL of chloroform, use this solution as the test solution. Separately, weigh 10 mg of Meprobamate RS, dissolve in 10 mL of chloroform, and use this solution as the standard solution. Spot 10 μL and 5 μL of the test solution and the standard solution, respectively, on the plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (4 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly, repeatedly by turns, with antimony (III) chloride TS and 3 % solution of furfural in chloroform until the black spot is developed, heat the plate at 110℃ for 15 minutes,the spot of meprobamate from test solution is not more than intense than the spot from standard solution (not more than 0.5 %).

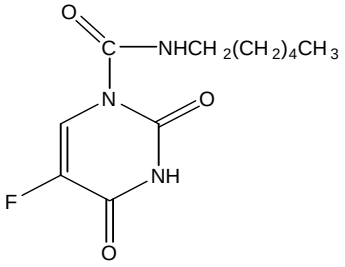
Loss on Drying Not more than 0.5 % (1 g, vaccum, 60 ºC, 3 hours).

Assay Weigh acculately about 0.4 g of Carisoprodol, add 10 mL of pyridine and 1 drop of phenolphthalein TS, and titrate with 0.1 mol/L sodium methoxide until pink color is developed. Add 25.0 mL of 0.1 mol/L sodium methoxide to this solution, heat for 30 minutes with reflux condenser and cool. Add 40 mL of ethanol (95) to this solution, and titrate with 0.1 mol/L hydrochloric acid VS(Indicator: 7 drops of phenolphthalein TS). Perform the blank, and make any necessary correction.

1 mL of 0.1 mol/L Sodium Methoxide
= 26.033 mg of $C_{12}H_{24}N_2O_4$.

Containers and Storage ***Containers***—Tight containers.

Carmofur



$C_{11}H_{l6}FN_3O_3$: 257.26

5-Fluoro-*N*-hexyl-2,4-dioxopyrimidine-1-carboxamide [*61422-45-5*]

Carmofur, when dried, contains not less than 98.0 % and not more than 101.0 % of carmofur ($C_{11}H_{l6}FN_3O_3$).

Description Carmofur is a white crystalline powder.

Carmofur is very soluble in *N,N*-dimethylformamide, freely soluble in acetic acid (100), soluble in ether, sparingly soluble in methanol or in ethanol (99.5) and

practically insoluble in water.

Melting point— About 111 ºC (with decomposition).

Identification (1) Proceed with 5 mg of Carmofur as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid and prepare the test solution: the test solution responds to the Qualitative Tests (2) for fluoride.

(2) Determine the absorption spectra of solutions of Carmofur and Carmofur RS in a mixture of methanol and phosphoric acid - acetic acid - boric acid buffer solution, (pH 2.0), (9 : 1) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavelength.

(3) Determine the infrared spectra of Carmofur and Carmofur RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Carmofur according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.2 g of Carmofur in 10 mL of a mixture of methanol and acetic acid (100) (99 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of methanol and acetic acid (100) (99 : 1) to make exactly 500 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 15 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene and acetone (5 : 3) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution. After exposure of the plate to bromine vapor for 30 seconds, spray evenly a solution of fluorescein in ethanol (95) (1 in 2500): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 50 ºC, 3 hours).

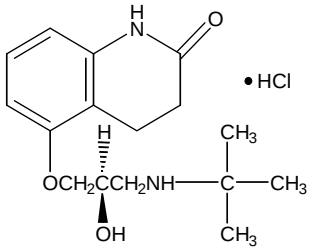
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Carmofur, previously dried, dissolve in 20 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide-methanol VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethyl-formamide TS).

Each mL of 0.1 mol/L tetramethylammonium hydroxide-methanol VS
= 25.726 mg of $C_{11}H_{16}FN_3O_3$

Containers and Storage ***Containers***—Tight containers.

Carteolol Hydrochloride



and enantiomer

$C_{16}H_{24}N_2O_3 \cdot HCl$: 328.83

5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydroquinolin-2-one hydrochloride [*51781-21-6*]

Carteolol Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$).

Description Carteolol Hydrochloride appears as white crystals or crystalline powder.

Carteolol Hydrochloride is soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95) or in acetic acid (100) and practically insoluble in ether.

The solution of Carteolol Hydrochloride (1 in 20) shows no optical rotation.

pH—Dissolve 1.0 g of Carteolol Hydrochloride in 100 mL of water: the pH of this solution is between 5.0 and 6.0.

Melting point—About 277 ºC (with decomposition).

Identification (1) Dissolve 0.1 g of Carteolol Hydrochloride in 5 mL of water and add 5 drops of Reinecke salt TS: a pale red precipitate is produced.

(2) Determine the absorption spectra of solutions of Carteolol Hydrochloride and Carteolol Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both sprectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the infrared spectra of Carteolol Hydrochloride and Carteolol Hydrochloride RS as directed in the potassium cholride disk method under Infrared Spectrophotometry: both spectra exhibit similar in tensities of absorption at the same wavenumbers.

(4) A solution of Carteolol Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH The pH of a solution of Carteolol Hydrochloride

(1 in 100) is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Carteolol Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Carteolol Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Carteolol Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Carteolol Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 2 mL of the test solution and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard soution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (50 : 20 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

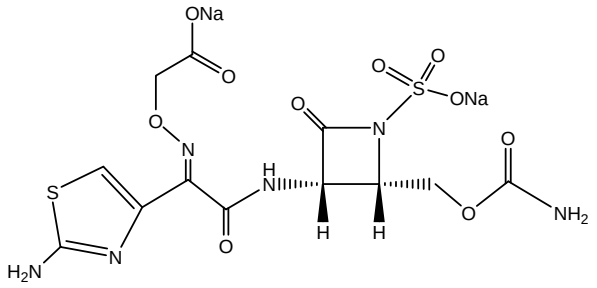
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.5 g of Carteolol Hydrochloride, previously dried, add 30 mL of acetic acid (100), dissolve by heating in a water-bath and cool. After adding 70 mL of acetic anhydride, titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.883 mg of $C_{16}H_{24}N_2O_3 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Carumonam Sodium



$C_{12}H_{12}N_6Na_2O_{10}S_2$: 510.37

Disodium 2-[(*Z*)-[1-(2-amino-1,3-thiazol-4-yl)-2-[[(2*S*,3*S*)-2-(carbamoyloxymethyl)-4-oxo-1-sulfonatoazetidin-3-yl]amino]-2-oxoethylidene]amino]oxyacetate [*86832-68-0*]

Carumonam Sodium contains not less than 850 μg (potency) and not more than 920 μg (potency) per mg of carumonam ($C_{12}H_{14}N_6O_{10}S_2$: 466.40), calculated on the anhydrous basis.

Description Carumonam Sodium appears as white to yellowish white crystals or crystalline powder.
Carumonam Sodium is freely soluble in water, soluble in formamide, very slightly soluble in methanol, and practically insoluble in ethanol (99.5) or in acetic acid (100).

Identification (1) Determine the absorption spectra of solutions of Carumonam Sodium and Carumonam Sodium (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Carumonam Sodium and Carumonam Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Carumonam Sodium in heavy water for nuclear magnetic resonance spectroscopy as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethyl-silylpropionate-*d*4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal, A, at around δ 5.5 ppm, and a single signal, B, at around δ 7.0 ppm. The ratio of the integrated intensity of each signal, A : B, is about 1 : 1.

(4) Carumonam Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +18.5 ~ +21.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Carumonam Sodium in 10 mL of water is between 5.0 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Carumonam Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Carumonam Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 15 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Carumonam Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) ***Related substance I***—Weigh accurately about 0.1 g of Carumonam Sodium, nd dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of related substances by the following equation: the amount of the related substance having the relative retention time of 0.7 with respect to the peak of carumonam is not more than 4.0 %, and the amount of each related substance other than the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 1.0 %.

$$\text{Amount (\%) of related substances} = \frac{\text{Amount [g (potency)] of Carumonam Sodium RS}}{W_T} \times \frac{A_T}{A_S}$$

W_T: Amount (g) of Carumonam Sodium taken

A_S: Peak area of carumonam from the standard solution

A_T: Area of each peak other than carumonam from the test solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13 % of the peak area of carumonam from the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 3 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of carumonam is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of carumonam.

(5) ***Related substance II***—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of related substances by the following equation: the amount of each related substance is not more than 1.0 %.

$$\text{Amount (\%) of related substances} = \frac{\text{Amount [g (potency)] of Carumonam Sodium RS}}{W_T} \times \frac{A_T}{A_S}$$

W_T: Amount (g) of Carumonam Sodium taken

A_S: Peak area of carumonam from the standard solution

A_T: Area of each peak other than carumonam from the test solution

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10000), methanol, and acetic acid (100) (74 : 25 : 1)

Flow rate: Dissolve 0.01 g of phthalic acid in the mobile phase to make 100 mL. Adjust the flow rate so that the retention time of phthalic acid is about 6.5 minutes when the procedure is run with 10 μL of this solution.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from this solution is equivalent to 7 to 13 % of the peak area of carumonam from the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of carumonam is not more than 2.0 %.

Time span of measurement: About 10 times as long as the retention time of carumonam

(6) ***Total related substances***—The total amount of related substances obtained in Related substances I and Related substances II is not more than 6.0 %.

Water Not more than 2.0 % (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (3 : 1) instead of methanol for water determination.)

Sterility Test It meets the requirement, when Carumonam Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.083 EU/mg (potency) of carumonam, when Carumonam Sodium is used in a sterile preparation.

Assay Weigh accurately about 40 mg (potency) each of Carumonam Sodium and Carumonam Sodium RS, and dissolve each in the mobile phase to make exactly 20 mL. To 5 mL each of these solutions add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of carumonam to that of the internal standard.

$$\text{Amount [μg (potency)] of carumonam } (C_{12}H_{14}N_6O_{10}S_2) = \text{Amount [μg (potency)] of Carumonam Sodium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of resorcinol in the mobile phase (9 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10000), methanol, and acetic acid (100) (97 : 2 : 1)

Flow rate: Adjust the flow rate so that the retention time of carumonam is about 10 minutes.

System suitability

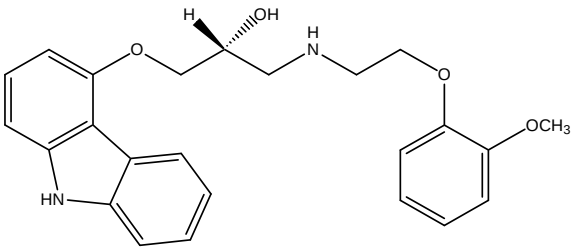
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of carumonam to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Carvedilol



and enantiomer

$C_{24}H_{26}N_2O_4$: 406.47

1-(9*H*-Carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy) ethylamino]propan-2-ol [*72956-09-3*]

Carvedilol, when dried, contains not less than 99.0 % and not more than 101.0 % of carvedilol ($C_{24}H_{26}N_2O_4$).

Description Carvedilol is a white crystalline powder. Carvedilol is slightly soluble in ethanol (95), soluble in dichloromethane, and practically insoluble in water. Carvedilol shows polymorphism.

Identification Determine the infrared spectra of Carvedilol and Carvedilol RS, previously dried, as directed in the potassium bromide disk method under

Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Carvedilol and Carvedilol RS in 2-propanol respectively, evaporate to dryness and repeat the test on the residues.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Carvedilol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances*** Dissolve 25 mg of Carvedilol, accurately weighed, in mobile phase to exactly 25.0 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add mobile phase to make exactly 100 mL, pipet 1.0 mL of this solution, add mobile phase to make exactly 10 mL and use this solution as the standard solution (1). Separately, dissolve 5 mg of carvedilol related substance I{(2*RS*-1-benzyl[2-(2-methoxy-phenoxy)ethyl]amino-3-(9*H*-carbazol-4-yloxy) propan-2-ol)} in 5.0 mL of the mobile phase, add the mobile phase to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 4.0 mL of the standard stock solution, add the mobile phase to make exactly 100 mL, pipet 1.0 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions. In the calculation of the amount of related substance II, multiply the peak area by 2, the correction factor. The peak area of related substance II obtained from the test solution is not larger than 2 times the area of the principal peak from the standard solution (1) (0.2 %), the peak area of related substance III from the test solution is not larger than 1.5 times the area of the principal peak from the standard solution (1) (0.15 %), the peak area of related substance I is not larger than that of related substance I from the standard solution (2) (0.02 %), and the peak area of any other related substance is not larger than the area of the principal peak from the standard solution (1) (0.1 %). The total area of the peaks other than related substance I from the test solution is not larger than 5 times the area of the principal peak from the standard solution (1) (not more than 0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak from the standard solution (1) (not more than 0.05 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilyl silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 55 ºC.

Mobile phase: To 1.77 g of potassium dihydrogen phosphate add water to make 650 ml, adjust to pH 2.0 with phosphoric acid and add 350 ml of acetonitrile.

Flow rate: 1.0 mL/minute.

Relative retention time: The relative retention times of related substances II, I, and III with respect to the retention time of carvedilol (about 4 minutes) are about 0.5, 2.9, and 3.8, respectively.

System suitability

System performance: Dissolve 5.0 mg of Carvedilol Related Substance I RS {(2*RS*)-1-benzyl[2-(2-methoxyphenoxy)ethyl]amino]-3-(9*H*-carbazol-4-yloxy)propan-2-ol]} in 5.0 mL of the test solution, and add the mobile phase to make exactly 100.0 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of carvedilol and related substance I is not less than 17. When the procedure is run with 20 μL of the standard solution (2) under the above operating conditions, the signal-to-noise ratio of the peak of related substance I is not less than 10.

Time span of measurement: About 6 times as long as the retention time of carvedilol

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.35 g of Carvedilol, previous dried, dissolve in 60 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.649 mg of $C_{24}H_{26}N_2O_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cefaclor Capsules

Cefaclor Capsules contain not less than 90.0 % and not more than 120 % of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81).

Method of Preparation Prepare as directed under Capsules, with Cefaclor Hydrate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Water Not more than 8.0 % (0.2 g, volumetric titration, direct titration).

Purity ***Related substances***—Weigh accurately the

contents of not less than 20 Cefaclor Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of cefaclor according to the labeled potency, dissolve in sodium dihydrogen phosphate solution (pH 2.5), sonicate if necessary, dilute with sodium dihydrogen phosphate (pH 2.5) to make exactly 10 mL, and use the filtrate as the test solution. Separately, prepare the standard solution as directed in related substances in the Purity under Cefaclor Hydrate, and use this solution as the standard solution. Proceed with 20 μL each of the test solution and standard solution as directed in related substances in the Purity under Cefaclor Hydrate, and calculate the amount (%) of each related substance (amount of each related substance: not more than 0.5 %, total amount of related substances: not more than 2.0 %).

Dissolution Test Perform the test with 1 capsule of Cefaclor Capsules at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Filter the dissolved solution 30 minutes after the start of the test. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 28 mg (potency) of Cefaclor RS, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed in the Assay under Cefaclor Hydrate, and determine the peak areas, A_T and A_S, of cefaclor in each solution. The dissolution rate of Cefaclor Capsules in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
= Amount [mg (potency)] of Cefaclor RS
$$\times \frac{A_T}{A_S} \times \frac{1}{C} \times 900$$

C: Labeled amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in 1 capsule

Uniformity of Dosage Units It meets the requirement of the Mass variation test.

Assay Weigh accurately the contents of not less than twenty Cefaclor Capsules, weigh accurately about 75 mg (potency) of the contents, according to the labeled potency, add the mobile phase to make exactly 250 mL, shake well, filter, and use the filtrate as the test solution. Perform the test as directed in the Assay under Cefaclor Hydrate.

Amount [μg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$$= \text{Amount [μg (potency)] of Cefaclor RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Cefaclor Extended-Release Tablets

Cefaclor Extended-Release Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81).

Method of Preparation Prepare as directed under Tablets, with Cefaclor Hydrate.

Identification The retention time of the principal peak in the chromatogram of the test solution corresponds to that in the chromatogram of the standard solution, as obtained in the Assay.

Water Not more than 6.5 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Cefaclor Extended-Release Tablets at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution solution. Take accurately a volume of the dissolved solution after 30 minutes from the start of the test, and add carefully the same volume of the dissolution solution, previously warmed to 37 ± 0.5 °C. Filter the dissolved solution through a membrane filter with a pore size not exceeding 0.5 μm. Pipet an amount of the filtrate, dilute with 0.1 mol/L hydrochloric acid TS so that each mL contains about 20 to 30 μg (potency), and use this solution as the test solution (30 minutes). Repeat this procedure after 60 minutes and after 240 minutes from the start of the test, and use the solutions thus obtained as test solutions (60 minutes, 240 minutes). Separately, weigh accurately about 0.11 g (potency) of Cefaclor RS, dissolve in 0.1 mol/L hydrochloric acid TS, dilute with 0.1 mol/L hydrochloric acid TS so that each mL contains about 20 to 30 μg (potency), and use this solution as the standard solution. Determine the absorbances at 265 nm of the test solutions and standard solution as directed under Ultraviolet-visible Spectrophotometry, using 0.1 mol/L hydrochloric acid TS as the blank, and calculate the dissolution rate. The dissolution rate of Cefaclor Extended-Release Tablets in 30 minutes, in 60 minutes, and in 240 minutes is 5 to 30 %, 20 to 50 %, and not less than 80 %, respectively.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test. Take 10 Cefaclor Extended-Release Tablets, dissolve in 25 mL of methanol for each tablet, and add the mobile phase so that each mL contains about 1.5 mg (potency) of cefaclor. Pipet 10 mL of this solution, add the internal standard solution to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 15 mg (potency) of Cefaclor RS, dissolve in the inter-

nal standard solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed in the Assay.

Assay Weigh accurately and powder not less than 20 Cefaclor Extended-Release Tablets. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) according to the labeled potency, dissolve in 150 mL of the internal standard solution, add the mobile phase to make 250 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 15 mg (potency) of Cefaclor RS, add 30 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefaclor to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) = \text{Amount [μg (potency)] of Cefaclor RS} \times \frac{Q_T}{Q_S} \times 5$$

Internal standard solution—Dissolve 0.45 g of sodium 2-naphthalenesulfonate in the mobile phase to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 250 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 1.0 g of sodium 1-pentanesulfonate in 780 mL of water, add 10 mL of triethylamine, mix, adjust the pH to 2.5 with phosphoric acid, and add 220 mL of methanol.

Flow rate: 1.5 mL/minute

Containers and Storage ***Containers***—Tight containers.

Cefaclor for Syrup

Cefacor for Syrup is a preparation for syrup, which is dissolved before use.

Cefaclor for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81).

Method of Preparation Prepare as directed under Syrups, with Cefaclor Hydrate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving Cefaclor for Syrup according to the label is between 2.5 and 5.0.

Purity ***Related substances***—Weigh accurately an amount of Cefaclor for Syrup, equivalent to 50 mg (potency) of cefaclor according to the labeled amount, add sodium dihydrogen phosphate solution (pH 2.5), shake carefully to avoid foaming, make exactly 50 mL, filter, and use the filtrate as the test solution. Use the test solution within 3 hours if stored at room temperature, and within 20 hours if stored in a refrigerator. Separately, weigh accurately 10 mg (potency) of Cefaclor RS, dissolve in sodium dihydrogen phosphate solution (pH 2.5) to make 20 mL, pipet 1 mL of this solution, add sodium dihydrogen phosphate solution (pH 2.5) to make 10 mL so that each mL contains 0.05 mg, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method. Calculate the amount of each related substance according to the following equation: the amount of each related substance is not more than 1.0 %, and the total amount of related substances is not more than 3.0 %. Exclude any related substance with an area less than 0.1 %.

$$\text{Amount (\%) of related substance} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration [mg (potency)/mL] of cefaclor in the standard solution

C_T: Concentration [mg (potency)/mL] of cefaclor in the test solution

A_i: Peak area of each related substance obtained from the test solution

A_S: Peak area of cefaclor obtained from the standard solution

Sodium dihydrogen phosphate solution (pH 2.5)—Dissolve 2.5 g of sodium dihydrogen phosphate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 6.9 g of sodium dihydrogen phosphate monohydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: A mixture of mobile phase A and acetonitrile (550 : 450)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	95	5
0-30	95→75	5→25
30 -45	75→0	25→100
45-55	0	100
55-60	0→95	100→5
60-70	95	5

Flow rate: 1.0 mL/minute

System suitability

System performance: Weigh accurately a suitable amount of cefaclor delta-3-isomer, dissolve in the standard solution to make a solution so that each mL contains 0.05 mg, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution under the above operating conditions, the retention time of the peak of cefaclor is between 23 and 29 minutes, the resolution between the peaks of cefaclor delta-3-isomer and cefaclor is not less than 2.0, and the symmetry factor of cefaclor is not more than 1.2.

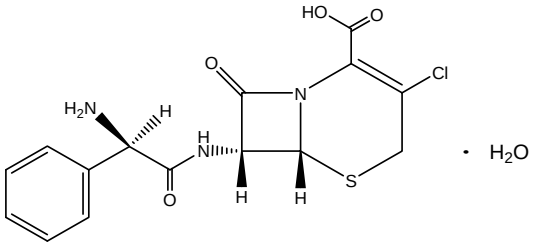
Water 2.0 % (0.5 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefaclor Hydrate. Weigh accurately an amount of Cefaclor for Syrup, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Cefaclor RS, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Cefaclor Hydrate



$C_{15}H_{14}ClN_3O_4S \cdot H_2O$: 385.82

(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacet]amido]-3-chloro-3,4-didehydrocepham-4-carboxylic acid monohydrate [*70356-03-5*]

Cefaclor Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of ($C_{15}H_{14}ClN_3O_4S$: 367.81), calculated on the anhydrous basis.

Description Cefaclor Hydrate appears as white to light yellow-white, crystals or crystalline powder, odorless or has a little of characteristic odor, and has a bit of bitter taste.

Cefaclor Hydrate is soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Cefaclor Hydrate and Cefaclor RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +105 ~ +120° (0.1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefaclor Hydrate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution by suspending 1.0 g of Cefaclor Hydrate in 10 mL of *N,N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 50 mg of Cefaclor Hydrate, dissolve in sodium dihydrogen phosphate TS (pH 2.5) to make exactly 10 mL, and use this solution as the test solution. Weigh accurately a suitable amount of Cefaclor RS, dissolve in sodium dihydrogen phosphate TS (pH 2.5) to a con-

stant concentration of about 0.05 mg/mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method and calculate the amount of each related substance: the amount of each related substance is not more than 0.5 %, and the amount of total related substances is not more than 2.0 %. If necessary, proceed with 20 μL of sodium dihydrogen phosphate (pH 2.5) in the same manner as the above to compensate the base line to make correction.

$$\text{Amount (\%) of each related substance} = \frac{C}{W} \times \frac{A_i}{A_S}$$

C: Concentration [μg (potency)/mL] of cefaclor in the standard solution

W: Amount [mg (potency)] of Cefaclor Hydrate taken

A_i: Peak area of each related substance obtained from the test solution

A_S: Peak area of cefaclor obtained from the standard solution

Sodium dihydrogen phosphate TS (pH 2.5)— Dissolve 2.5 g of sodium dihydrogen phosphate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Mobile phase A: Dissolve 6.9 g of sodium dihydrogen phosphate monohydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: A mixture of mobile phase A and acetonitrile (550 : 450)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	95	5
0-30	95→75	5→25
30-45	75→0	25→100
45-55	0	100
55-60	0→95	100→5
60-70	95	5

Flow rate: 1.0 mL per minute.

System suitability

Dissolve a suitable amount of Cefaclor Delta-3 Isomer RS in the standard solution so that each mL contains about 0.05 mg, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution under the above operating conditions, the peak of cefaclor is eluted between 23 and 29 minutes, the resolution between the peaks of cefaclor delta-3 isomer and cefaclor is not less than 2.0, and the symmetry factor of the peak of cefaclor is not more than 1.2.

pH The pH of a solution obtained by dissolving 0.25 g of Cefaclor Hydrate in 10 mL of water is between 3.0 and 4.5.

Water Not less than 3.0 and not more than 8.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 15 mg (potency) each of Cefaclor Hydrate and Cefaclor Hydrate RS, dissolve each in a suitable amount of the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and obtain the peak areas of cefaclor, A_T and A_S.

$$\text{Amount [μg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) = \textit{Amou}\text{nt [μg (potency)] of Cefaclor RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: Weigh 1.0 g of sodium 1-pentanesulfonate, dissolve in 750 mL of water, add 10 mL of triethylamine, mix well, and adjust the pH to 2.5 ± 1 with phosphoric acid. To this solution add 220 mL of methanol.

Flow rate: 1.5 mL/minute

System suitability

System performance: When the procedure is run with the system suitability solution under the above operating conditions, the relative retention times of cefaclor and cefaclor delta-3 isomer are about 0.8 and about 1.0, respectively, and the resolution between the peaks of cefaclor and cefaclor delta-3 isomer is not less than 2.5 with the symmetry factor being not more than 1.5.

System repeatability: When the test is repeated 5 times with the system suitability solution under the above operating conditions, the relative standard deviation of the peak of cefaclor is not more than 2 %.

System suitability solution—Weigh accurately a suitable amount each of Cefaclor Hydrate and Cefaclor Delta-3 Isomer RS, and dissolve in the mobile phase so that each mL contains 0.3 mg.

Containers and Storage ***Containers***—Tight containers.

Cefadroxil Capsules

Cefadroxil Capsules contain not less than 95.0 % and not more than 105.0 % of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of Preparation Prepare as directed under Capsules, with Cefadroxil Hydrate.

Identification Dissolve the contents of Cefadroxil Capsules, equivalent to 10 mg (potency) of cefadroxil according to the labeled amount, in 500 mL of water, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nmm.

Water Not more than 7.0 % (0.15 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Cefadroxil Capsules at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution solution. Take not less than 20 mL of the dissolved solution 90 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of cefadroxil according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil RS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 263 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. The dissolution rate of Cefadroxil Capsules in 90 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$)
= Amount [mg (potency)] of Cefadroxil RS

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

C: Labeled amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$) in 1 capsule

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test. Place 1 capsule of Cefadroxil Capsules in 300 mL of water, disperse with the aid of ultrasonic waves, shake for 30 minutes, and add water to make exactly 500 mL. Pipet 5 mL of this solution, and add water to make a solution so that each mL contains 0.1 mg (potency) of cefadroxil. Filter the solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil RS, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil Hydrate.

Assay Weigh accurately the contents of not less than 20 Cefadroxil Capsules. Weigh accurately a portion of the contents, equivalent to about 50 mg (potency) of cefadroxil, add 30 mL of water, shake for 30 minutes, then add water to make exactly 500 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil RS, add water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil for Syrup.

Amount [μg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
= Amount [μg (potency)] of Cefadroxil RS

$$\times \frac{A_T}{A_S} \times \frac{5}{2}$$

Containers and Storage ***Containers***—Tight containers.

Cefadroxil for Syrup

Cefadroxil for Syrup is a preparation for syrup, which is suspended before use.
Cefadroxil for Syrup contains not less than 95.0 % and not more than 110.0 % of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of Preparation Prepare as directed under Syrups, with Cefadroxil.

Identification Dissolve an amount of Cefadroxil for Syrup, equivalent to 10 mg (potency) of cefadroxil according to the labeled amount, in 500 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

pH The pH of a solution prepared by dissolving Cefadroxil for Syrup according to the label is between 4.5 and 6.0.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Dissolution Test Weigh accurately an amount of Cefadroxil for Syrup, equivalent to about 0.1 g (potency) of cefadroxil according to the labeled amount, and perform the test at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution (put the sample in the dissolution solution so that it disperses). Take not less than 20 mL of the dissolved solution 15 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Cefadroxil RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 263 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. The dissolution rate of Cefadroxil for Syrup in 15 minutes is not less than 85 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cefadroxil } (C_{16}H_{17}N_3O_5S) = \frac{\text{Amount [mg (potency)] of Cefadroxil RS}}{\text{Amount (g) of Cefadroxil for Syrup taken}} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 450$$

C: Labeled amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$) in 1 g

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately an amount of Cefadroxil for Syrup, equivalent to about 50 mg (potency) of cefadroxil according to the labeled amount, dissolve in water to make exactly 500 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil RS, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefadroxil in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefadroxil } (C_{16}H_{17}N_3O_5S) = \text{Amount [μg (potency)] of Cefadroxil RS} \times \frac{A_T}{A_S} \times \frac{5}{2}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 262 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (17 in 12500) and methanol (17 : 3).

Flow rate: Adjust the flow rate so that the retention time of cefadroxil is about 5 minutes.

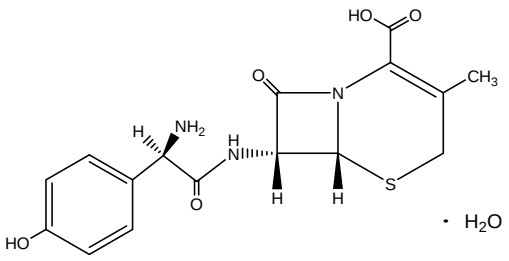
System suitability

System performance: Dissolve 5 mg (potency) of cefadroxil and 10 mg (potency) of cefatrizine propylene glycol in 50 mL of water. When the procedure is run with 10 μL of this solution under the above operatin conditions, cefadroxil and cefatrizine propylene glycol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefadroxil is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefadroxil Hydrate



$C_{16}H_{17}N_3O_5S \cdot H_2O$: 381.40

(6*R*,7*R*)-7-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acet]amido]-3-methyl-3,4-didehydrocepham-4-carboxylic acid monohydrate
[*66592-87-8*]

Cefadroxil Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39), calculated on the anhydrous basis

Description Cefadroxil Hydrate is a white to pale yellowish white powder.
Cefadroxil Hydrate is sparingly soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectra

of the solutions of Cefadroxil Hydrate and Cefadroxil Hydrate RS in ethanol (1 in 50000), as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefadroxil Hydrate and Cefadroxil Hydrate RS, as directed in the potassium bromide disk method under the Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefadroxil Hydrate in a mixture of heavy water for nuclear magnetic resonance spectroscopy and hydrochloric acid for nuclear magnetic resonance spectroscopy (3 : 1) (1 in 10), using sodium 3-(trimethylsilyl)propionate-d_4 for nuclear magnetic resonance spectroscopy as an internal standard, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits a single signal A at around δ 2.1 ppm, a double signal B at around δ 7.0 ppm, and a double signal C at around δ 7.5 ppm. The ratio of integrated intensity of each signal, A : B : C, is about 3 : 2 : 2.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +164 ~ +182° (0.6 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefadroxil Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.1 g of Cefadroxil Hydrate in 4 mL of a mixture of ethanol (99.5), water, and diluted hydrochloric acid (1 in 5) (75 : 22 : 3), and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of ethanol (99.5), water, and diluted hydrochloric acid (1 in 5) (75 : 22 : 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate if silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water, ethanol (99.5), and formic acid (14 : 5 : 5 : 1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100 °C for 10 minutes: the spots other than the principal spot obtained from the test solution are not more intense than the spot from the standard solution.

(3) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cefadroxil Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS, add 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Cefadroxil Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

pH The pH of a solution obtained by dissolving 1.0 g of Cefadroxil Hydrate in 200 mL of water is between 4.0 and 6.0.

Water Not less than 4.2 % and not more than 6.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 50 mg of Cefadroxil Hydrate and Cefadroxil Hydrate RS, dissolve each in a suitable amount of the water, then add water to make exactly 500 mL, and use these solutions as the test solution and standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefadroxil.

$$\text{Amount [μg (potency)] of cefadroxil } (C_{16}H_{17}N_3O_5S) = \text{Amount [μg (potency)] of Cefadroxil RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 250 mm in length, packed

with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40 °C.
Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (17 in 12500) and methanol (17 : 3).
Flow rate: Adjust the flow rate so that the retention time of cefadroxil is about 5 minutes.
System suitability

System performance: Dissolve about 5 mg (potency) of cefadroxil and about 10 mg (potency) of cefatrizine propylene glycol in 50 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefadroxil is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefadroxil Tablets

Cefadroxil Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of Preparation Prepare as directed under Tablets, with Cefadroxil Hydrate.

Identification Dissolve separately about 50 mg (potency) each of powdered Cefadroxil Tablets and Cefadroxil RS in a mixture of acetone and 0.1 mol/L hydrochloric acid TS (40 : 10) to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, benzene, water, and acetic acid (100) (100 : 20 : 20 : 10). Spray evenly a solution of 0.2 g of ninhydrin in citric acid-acetate buffer solution to make 100 mL on the plate: the spots obtained from the test solution and standard solution show the same R_f value.

Water Not more than 8.0 % (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Cefadroxil Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 m L of water as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Cefadroxil RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 263 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Cefadroxil Tablets in 30 minutes is not less than 75 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount of cefadroxil } (C_{16}H_{17}N_3O_5S) = C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder 20 Cefadroxil Tablets. Weigh accurately a portion of the powder, equivalent to about 0.2 g (potency) according to the labeled potency, dissolve in pH 5.0 phosphate buffer solution to make exactly 200 mL, and use this solution as the test solution. Separately, weigh accurately about 0.2 g (potency) of Cefadroxil RS, dissolve in pH 5.0 phosphate buffer solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefadroxil in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefadroxil } (C_{16}H_{17}N_3O_5S) = \text{Amount [μg (potency)] of Cefadroxil RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.0 to 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: A mixture of pH 5.0 phosphate buffer solution and acetonitrile (96 : 4).

Flow rate: 1.5 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates is

not less than 1800, the capacity factor is between 2.0 and 3.5, and the symmetry factor is not more than 2.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefadroxil is not more than 2.0 %.

Phosphate buffer solution pH 5.0—Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 2000 mL, and adjust the pH to 5.0 with 10 mol/L potassium hydroxide.

Containers and Storage ***Containers***—Tight containers.

Cefalexin Capsules

Cefalexin Capsules contain not less than 93.0 % and not more than 107.0 % of the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of Preparation Prepare as directed under Capsules, with Cefalexin Hydrate.

Identification Take out the contents of Cefalexin Capsules, to a quantity of the contents, equivalent to 70 mg (potency) of cefalexin according to the labeled amount, add 25 mL of water, shake vigorously for 5 minutes, and filter. To 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 260 nm and 265 nm.

Water Not more than 10.0 % (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Cefalexin Capsules at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 60 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of cefalexin ($C_{16}H_{17}N_3O_4S$), and use this solution as the test solution. Separately, weigh accurately about 22 mg (potency) of Cefalexin RS, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 262 of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Cefalexin Capsules in 60 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$)
= Amount [mg (potency)] of Cefalexin RS

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 capsule

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Open 1 capsule of Cefalexin Capsules, add $3V/5$ mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 1.25 mg (potency) of cefalexin. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg (potency) of Cefalexin RS, and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard in each solution.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)

$$= W_S \times \frac{Q_T}{Q_S} \times \frac{V}{20}$$

W_S: Amount [mg (potency)] of Cefalexin RS

Assay Take out the contents of not less than 20 capsules of Cefalexin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add phosphate buffer solution (pH 4.5) to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the test solution. Then, proceed as directed in the Assay under Cefalexin for Syrup.

Containers and Storage ***Containers***—Tight containers.

Cefalexin for Syrup

Cefalexin for Syrup is a preparation for syrup, which is dissolved or suspended before use.
Cefalexin for Syrup contains not less than 90.0 % and not more than 110.0 % of the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of Preparation Prepare as directed under Syrups, with Cefalexin Hydrate.

Identification Dissolve an amount of Cefalexin for Syrup, equivalent to 3 mg (potency) of cefalexin according to the labeled amount, in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 260 nm and 264 nm.

pH The pH of a solution obtained by dissolving Cefalexin for Syrup according to the label is between 3.0 and 6.0.

Water Not more than 5.0 % (0.4 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) Perform the test according to the following method: it meets the requirement of the Content uniformity test.
Take out the total contents of 1 pack of Cefalexin for Syrup, add 3*V*/5 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly *V* mL so that each mL contains about 1 mg (potency) of cefalexin, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the tst soution. Then, proceed as directed in the Assay.

$$\text{Amount [mg (potency)] of cefalexin } (C_{16}H_{17}N_3O_4S) = W_S \times \frac{Q_T}{Q_S} \times \frac{V}{20}$$

W_S: Amount [mg (potency)] of Cefalexin RS

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15000)

Dissolution Test Weigh accurately an amount of Cefalexin for Syrup, equivalent to about 0.25 g (potency) of cefalexin according to the labeled amount, and perform the test at 50 revolutions per minute according to Method 2, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 15 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, and determine the absorbances, A_T and A_S, at 262 nm. The dissolution rate of Cefalexin for Syrup in 15 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cefalexin } (C_{16}H_{17}N_3O_4S) = \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 1125$$

W_S: Amount [mg (potency)] of Cefalexin RS
W_T: Amount (g) of Cefalexin for Syrup taken
C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 g

Assay Powder Cefalexin for Syrup, if necessary, and weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of cefalexin } (C_{16}H_{17}N_3O_4S) = \text{Amount [μg (potency)] of Cefalexin RS} \times \frac{Q_T}{Q_S} \times 5$$

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.0 mm in internal diameter and about 75 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with dilute phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 6 minutes.

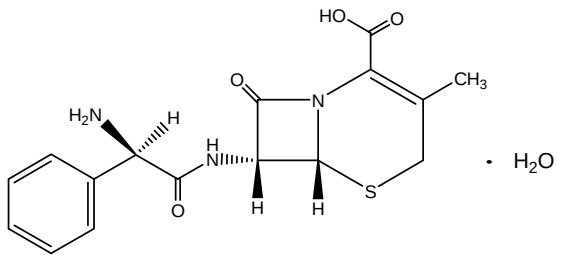
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefalexin Hydrate



$C_{16}H_{17}N_3O_4S \cdot H_2O$: 365.40

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetamido]-3-methyl-3,4-didehydrocepham-4-carboxylic acid monohydrate [*23325-78-2*]

Cefalexin Hydrate contains not less than 950 μg (potency) and not more than 1030 μg (potency) per mg of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39), calculated on the anhydrous basis.

Description Cefalexin Hydrate appears as white to light yellowish white crystals or crystalline powder.
Cefalexin Hydrate is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) or in *N,N*-dimethylformamide.
Cefalexin Hydrate is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Cefalexin Hydrate and Cefalexin RS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefalexin Hydrate and Cefalexin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the 1H spectrum of a solution of Cefalexin Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 200) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropane-sulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.8 ppm, and a single or a sharp multiple signal B at around δ 7.5 ppm. The ratio of integrated intensity of these signals, A : B, is about 3 : 5.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +144 ~ +158° (0.125 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.5 g of Cefalexin Hydrate in 10 mL of water is between 3.0 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefalexin Hydrate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Cefalexin Hydrate by suspending in 10 mL of *N,N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cefalexin Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

Content (ppm) of dimethylaniline

$$= \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Cefalexin Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

(4) ***Related substances***—Dissolve about 25 mg of Cefalexin Hydrate in a solution of potassium dihydrogenphosphate (9 in 500) to make 5 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the areas of each peak by the automatic integration method. If necessary, correct the change of the base-line due to the potassium dihydrogenphosphate solution by proceeding in the same manner with 20 μL of a solution of potassium dihydrogenphosphate (9 in 500): each peak area other than cefalexin from the test solution is not larger than the peak area of cefalexin from the standard solution, and the total of the peak areas which are larger than 1/50 times the peak area of cefalexin from the standard solution and those other than cefalexin from the test solution is not larger than 5 times the peak area of cefalexin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 1.0 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.

Mobile phase B: Dissolve 1.0 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid. To this solution add 350 mL of acetonitrile and 350 mL of methanol.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	100	0
0-1	100	0
1-34.5	100→0	0→100
34.5-35.5	0	100

Flow rate: 1.0 mL/minute

System suitability

Detection sensitivity: Pipet 2 mL of the standard solution, and add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL. Confirm that the peak area of cefalexin obtained from 20 μL of this solution under the above operating conditions is equivalent to 1.8 to 2.2 % of that of cefalexin obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefalexin are not less than 150000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL each of the standard solution under the above operating conditions, the relative stnandard deviation of the retention time and the peak areas of cefalexin are not more than 2.0 %, respectively.

Time span of measurement: About 2 times as long as the retention time of cefalexin beginning after the solvent peak.

Water 4.0 % ~ 8.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g (potency) each of Cefalexin Hydrate and Cefalexin RS, dissolve each in 0.1 mol/L phosphate buffer solution (pH 4.5), and add water to make exactly 100 mL. Pipet 10 mL each of these solutions, add 5.0 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

Amount [μg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
= Amount [μg (potency)] of Cefalexin Hydrate RS

$$\times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 1500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 1000 mL of water, adjust to pH 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 7 minutes.

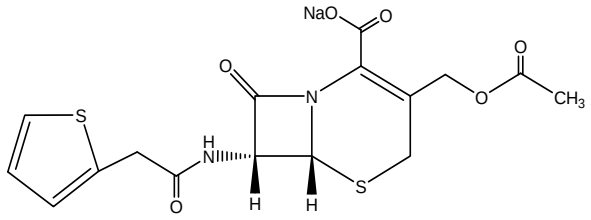
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefalexin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefalotin Sodium



$C_{16}H_{15}N_2NaO_6S_2$: 418.42

Sodium (6*R*,7*R*)-7-[2-(thiophen-2yl)acetamido]-3-acetyloxymethyl-3,4-didehydrocepham-4-carboxylate [*58-71-9*]

Cefalotin Sodium contains not less than 920 μg (potency) and not more than 980 μg (potency) per mg of cefalotin ($C_{16}H_{16}N_2O_6S_2$: 396.44), calculated on the anhydrous basis.

Description Cefalotin Sodium appears as white to pale yellowish white crystals or crystalline powder.
Cefalotin Sodium is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) Determine the absorption spectra of solutions of Cefalotin Sodium and Cefalotin Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefalotin Sodium and Cefalotin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefalotin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance imaging as an internal reference compound: it exhibits a single signal A at around δ 2.1 ppm, a single or sharp multiple signal B at around δ 3.9 ppm, and a multiple signal C at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A : B : C, is about 3 : 2 : 2.

(4) Cefalotin Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +124 ~ +134° (5.0 g, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Cefalexin Hydrate in 10 mL of water is between 4.5 and 7.5.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Cefalotin Sodium in 10 mL of water: the solution is clear. The absorbance of this solution at 450 nm, determined as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.20.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefalotin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefalotin Sodium according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Pipet 1 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution obtained in the Assay and the standard solution prepared here as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefalotin from the test

solution is not larger than the peak area of cefalotin from the standard solution, and the total area of the peaks other than cefalotin from the test solution is not larger than 3 times the peak area of cefalotin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Detection sensitivity: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefalotin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the standard solution.

System performance: Heat the standard solution at 90 °C for about 10 minutes, and cool. Pipet 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin, is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 3 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 2.0 %.

Time span of measurement: About 4 times as long as the retention time of cefalotin.

Water Not more than 1.0 % (0.5 g, volumetric titration, back titration)

Sterility Test It meets the requirement, when Cefalotin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.13 EU/mg (potency) of cefalotin, when Cefalotin Sodium is used in a sterile preparation.

Assay Weigh accurately about 20 mg (potency) each of Cefalotin Sodium and Cefalotin Sodium RS, dissolve each in the mobile phase to make exactly 20 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of cefalotin of these solutions.

$$\text{Amount [μg (potency)] of cefalotin } (C_{16}H_{16}N_2O_6S_2) = \textit{Amou}\text{nt [μg (potency)] of Cefalotin Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 17 g of sodium acetate trihydrate in 790 mL of water, and add 0.6 mL of acetic acid (100). If necessary, adjust the pH to 5.9 with 0.1 mol/L sodium hydroxide TS or acetic acid (100). To this solution add 150 mL of acetonitrile and 70 mL of ethanol (95).

Flow rate: Adjust the flow rate so that the retention time of cefalotin is about 12 minutes.

System suitability

System performance: Heat the standard solution in a water bath of 90 °C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operation conditions, the resolution between peak of cefalotin and the peak, having the relative retention time of 0.5 with respect to cefalotin is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers

Cefalotin Sodium for Injection

Cefalotin Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefalotin Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefalotin ($C_{16}H_{16}N_2O_6S_2$: 396.44).

Method of Preparation Prepare as directed under Injections, with Cefalotin Sodium.

Description Cefalotin Sodium for Injection appears as white to pale yellowish white powder.

Identification Determine the absorption spectra between 220 nm and 310 nm of 0.0025 % solutions of Cefalotin Sodium for Injection and Cefalotin Sodium RS as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima and minima at the same wavelengths.

pH The pH of a solution obtained by dissolving an amount of Cefalotin Sodium for Injection, equivalent to 0.25 g (potency) of cefalotin, in 1 mL of water is between 5.0 and 7.5.

Water Not more than 1.5 % (1.0 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.13 EU/mg (potency) of cefalotin.

Foreign Insoluble Matter Test It meets the requirement.

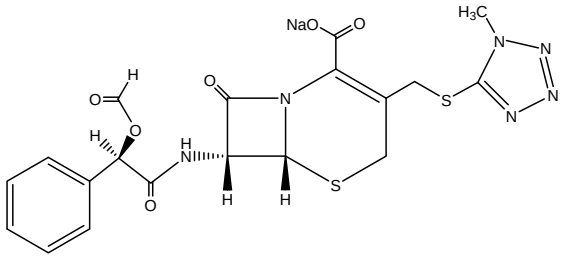
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefalotin Sodium. Weigh accurately a suitable amount of Cefalotin Sodium for Injection according to the labeled potency, dissolve in the mobile phase to make a solution so that each mL contains 1 mg (potency), and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefamandole Nafate



$C_{19}H_{17}N_6NaO_6S_2$: 512.50

Sodium (6*R*,7*R*)-7-[[(2*R*)-2-formyloxy-2-phenylacet]amido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*42540-40-9*]

Cefamandole Nafate contains not less than 810 μg (potency) and not more than 1000 μg (potency) per mg of cefamandole ($C_{18}H_{18}N_6O_5S_2$: 462.51), calculated on the anhydrous basis

Description Cefamandole Nafate is a white powder. Cefamandole Nafate is soluble in water or in ethanol, and very slightly soluble in ethylacetate, in chloroform, in ether or in benzene.

Identification Dissolve separately 10 mg each of Cefamandole Nafate and Cefamandole Nafate RS in the developing solvent to make 1 mL, and use these solutions as the test solution and standard solution. Use these solutions promptly after preparation. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, acetic acid (100), and water (5 : 2 : 1 : 1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution and standard solution show the same R_f value.

pH The pH of a solution obtained by dissolving 1 g of Cefamandole Nafate in 10 mL of water is between 3.5 and 7.0.

Purity ***Heavy metals***—Proceed with 1.0 g of Cefamandole Nafate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cefamandole Nafate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of cefamandole, when Cefamandole Nafate is used in a sterile preparation.

Assay ***The Standard curve method*** (1) Culture medium Agar media for seed and base layer- Use the medium in I 2 1) (3) ① or ② under Microbial Assay for Antibiotics.

(2) Test organism- *Staphylococcus aureus* ATCC 6538P.

(3) Weigh accurately an appropriate amount of Cefamandole Nafate, dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution so that each mL contains 1 mg (potency), hydrolyze at 37 °C for 60 minutes in water bath, and pipet an appropriate amount of this solution and dilute in 1 % phosphate buffer solution, pH 6.0 to make solution so that each mL contains 2.00 μg (potency) and use this solution as the test solution. Separately, weigh accurately an appropriate amount of Cefamandole Nafate RS, dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution so that each mL contains 1 mg (potency), and allow to stand at 37 ºC in water bath for 60 minutes. Keep the standard stock solution at not exceeding 5 ºC and use within one day. Pipet an appropriate amount of this solution and dilute in 1 % phosphate buffer solution, pH 6.0 to make solutions so that

each mL contains 1.28, 1.60, 2.00, 2.50, and 3.12 μg (potency), and use these solutions as the standard solutions. Also use the solution containing 2.00 μg (potency) per mL as the standard mid-diluted solution. Perform the test with these solutions according to the Standard curve method (II 4) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Cefamandole Nafate for Injection

Cefamandole Nafate for Injection is a preparation for injection, which is dissolved before use.
Cefamandole Nafate for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefamandole ($C_{18}H_{18}N_6O_5S_2$: 462.51).

Method of Preparation Prepare as directed under Injections, with Cefamandole Nafate.

Description Cefamandole Nafate for Injection appears as white powder.

Identification Proceed as directed in Identification under Cefamandole Nafate.

pH Dissolve an amount of Cefamandole Nafate for Injection, equivalent to 0.1 g (potency) of cefamandole, in 1 mL of water, and allow to stand for 30 minutes: the pH of this solution is between 6.0 and 8.0.

Water Not more than 3.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of cefamandole.

Foreign Insoluble Matter Test It meets the requirement.

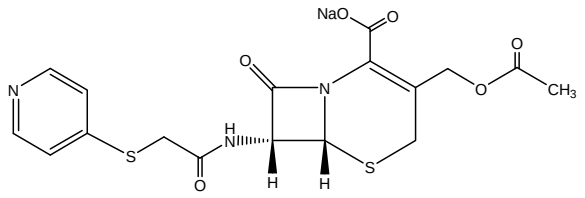
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Standard curve method*** Proceed as directed in the Assay under Cefamandole Nafate. Weigh accurately a suitable amount of Cefamandole Nafate for Injection according to the labeled potency, dissolve in 0.1 mol/L phosphate buffer solution (pH 8.0) to make a solution so that each mL contains about 1 mg (potency), and hydrolyze in a water bath at 37 °C for 60 minutes. Pipet a suitable amount of this solution, add 1 % phosphate buffer solution (pH 6.0) to render the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefapirin Sodium



$C_{17}H_{16}N_3NaO_6S_2$: 445.45

Sodium (6*R*,7*R*)-7-[2-(pyridin-4-yl)sulfanylacetamido]- 3-acetyloxymethyl-3,4-didehydrocepham-4-carboxylate [*24356-60-3*]

Cefapirin Sodium contains not less than 865 μg (potency) per mg of cefapirin ($C_{17}H_{17}N_3O_6S_2$: 423.46), calculated on the anhydrous basis.

Description Cefapirin Sodium is a white to yellowish white powder.
Cefapirin Sodium is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in acetone.

Identification (1) Determine the absorption spectra of solutions of Cefapirin Sodium and Cefapirin Sodium RS (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefapirin Sodium and Cefapirin Sodium RS, as directed in the potassium bromide disk method under the Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the ^1H spectrum of a solution of Cefapirin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-(trimethyl-silyl)propionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits a single signal A at around δ 2.2 ppm, and multiple signals B and C at around δ 7.3 ppm and around δ 8.3 ppm, respectively. The ratio of the area of each signal, A : B : C, is 3 : 2 : 2.

(4) Cefapirin Sodium responds to the Qualitative Tests 1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +157 ~ +175° (2 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.1 g of Cefapirin Sodium in 10 mL of water is between 6.5 and 8.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefapirin Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Cefapirin Sodium according to Method 3 and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 25) (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.1 g of Cefapirin Sodium in a mixture of acetone and water (3 : 1) to make exactly 5 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of acetone and water (3 : 1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water, and acetic acid (100) (5 : 2 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the test solution are not more intense than the spot from the standard solution.

Water Not more than 2.0 % (0.7 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefapirin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.17 EU/mg (potency) of cefapirin, when Cefapirin Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Cefapirin Sodium and Cefapirin Sodium RS, dissolve each in 1 % phosphate buffer solution, pH 6.0 to make exactly 100 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution and 1 % phosphate buffer solution, pH 6.0 to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefapirin to that of the internal standard.

Amount [μg (potency)] of cefapirin ($C_{17}H_{17}N_3O_6S_2$)
= Amount [μg (potency)] of Cefapirin Sodium RS

$$\times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of vanillin (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 2.6) and acetonitrile (93 : 7).

Flow rate: Adjust the flow rate so that the retention time of cefapirin is about 7 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, cefapirin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefapirin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Cefapirin Sodium for Injection

Cefapirin Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefapirin Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefapirin ($C_{17}H_{17}N_3O_6S_2$: 423.47).

Method of Preparation Prepare as directed under Injections, with Cefapirin Sodium.

Identification Dissolve separately 10 mg (potency) each of Cefapirin Sodium for Injection and Cefapirin Sodium RS in 10 mL of methanol, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water, and methanol (4 : 3 : 2). Spray evenly 0.1 mol/L potassium permanganate TS on the plate: the yellow spots obtained from the test solution and standard solution show the same R_f value.

pH The pH of a solution obtained by dissolving an amount of Cefapirin Sodium for Injection, equivalent to 1.0 g (potency) of cefapirin, in 100 mL of water is between 6.5 and 8.5.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.17 EU/mg (potency) of cefapirin.

Foreign Insoluble Matter Test It meets the requirement.

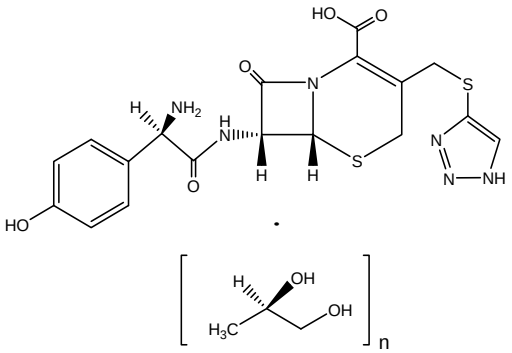
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefapirin Sodium. Weigh accurately an amount of Cefapirin Sodium for Injection, equivalent to about 0.1 g (potency) according to the labeled potency, and dissolve in 1 % phosphate buffer solution (pH 6.0) to make exactly 100 mL. Pipet 5 mL of this solution, add 5 mL of the internal standard solution, add 1 % phosphate buffer solution (pH 6.0) to make exactly 100 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefatrizine Propylene Glycol



$C_{18}H_{18}N_6O_5S_2 \cdot (C_3H_8O_2)n$

(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acet]amido]-3-[(2H-triazol-4-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylic acid (2*R*)-propane-1,2-diol [*51627-14-6*, Cefatrizine]

Cefatrizine Propylene Glycol contains not less than 816 μg (potency) and not more than 876 μg (potency) per mg of cefatrizine ($C_{18}H_{18}N_6O_5S_2$: 462.50), calculated on the anhydrous basis.

Description Cefatrizine Propylene Glycol is a white to yellowish white powder.
Cefatrizine Propylene Glycol is sparingly soluble in water, and practically insoluble in methanol or in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cefatrizine Propylene Glycol and Cefatrizine Propylene Glycol RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefatrizine Propylene Glycol and Cefatrizine Propylene Glycol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefatrzine Propylene Glycol in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy (3 : 1) (1 in 10), using sodium 3-(trimethyl-silyl)propionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits a double signal A at around δ 1.2 ppm, a double signal B at around δ 7.0 ppm, a double signal C at around δ 7.5 ppm and a single signal D at around δ 8.3 ppm. The ratio of integrated intensity of these signals, A : B : C : D, is about 3 : 2 : 2 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +52 ~ +58° (2.5 g calculated on the anhydrous basis, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.1 g of Cefatrizine Propylene Glycol in 10 mL of water is between 3.5 and 6.0.

Absorbance $E_{1cm}^{1\%}$ (270 nm): 190 ~ 220 (40 mg, water, 2000 mL).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefatrizine Propylene Glycol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Cefatrizine Propylene Glycol according to Method 3 and perform the test (not more than 2 ppm). Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 25).

(3) ***Related substances***—Dissolve 25 mg of Cefatrizine Propylene Glycol in 5 mL of water, and use this solution as the test solution. Pipet 1 mL of the test

solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100 °C for 10 minute: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible)

Assay Weigh accurately about 50 mg (potency) each of Cefatrizine Propylene Glycol and Cefatrizine Propylene Glycol RS, dissolve each in water to make exactly 25 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and calculate the peak areas, A_T and A_S, of cefatrizine of these solutions.

$$\text{Amount [μg (potency)] of cefatrzine } (C_{18}H_{18}N_6O_5S_2) = \text{Amount [μg (potency)] of Cefatrizine Propylene Glycol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: Dissolve 2.04 g of potassium dihydrogenphosphate in water to make 1500 mL, and add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefatrizine is about 6 minutes.

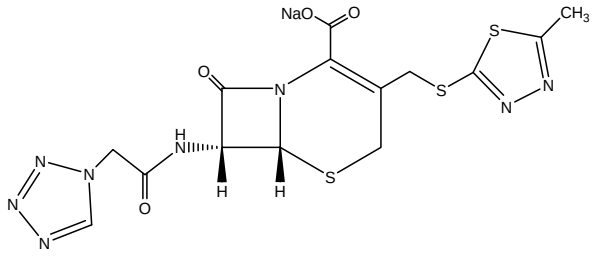
System suitability

System performance: Weigh accurately about 5 mg (potency) of Cefadroxil and about 10 mg of Cefatrizine Propylene Glycol, dissolve in 50 mL of water. When the procedure is run with 10 μL of the solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefatrizine are not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$: 476.49

Sodium (6*R*,7*R*)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-7-[[2-(tetrazol-1-yl)acet]amido]-3,4-didehydrocepham-4-carboxylate [*27164-46-1*]

Cefazolin Sodium contains not less than 900 μg (potency) and not more than 975 μg (potency) per mg of ($C_{14}H_{14}N_8O_4S_3$: 454.51), calculated on the anhydrous basis.

Description Cefazolin Sodium appears as white to light yellowish white crystals or crystalline powder.
Cefazolin Sodium is freely soluble in water or in formamide, slightly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cefazolin Sodium and Cefazolin Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefazolin Sodium and Cefazolin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefazolin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethyl-silylpropionate-d_4 for nuclear magnetic resonance spectroscopy as the internal standard, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals, A and B, at around δ 2.7 ppm and at around δ 9.3 ppm, respectively. The ratio of integrated intensity of these signals, A : B, is about 3 : 1.

(4) Cefazolin Sodium responds to the Qualitative Tests 1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -19 ~ -23° (2.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1 g of Cefazolin Sodium in 10 mL of water is between 4.8 and 6.5.

Absorbance $E_{1\,cm}^{1\%}$ (272 nm): 264 ~ 292 (1.6 mg, water, 100 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefazolin Sodium in 10 mL of water: the solution is clear and colorless to pale yellow, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry is not more than 0.35. The test should be performed within 10 minutes after preparing the solution.

(2) ***Heavy metals***—Proceed with 2.0 g of Cefazolin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Cefazolin Sodium according to Method 3, and perform the test. When preparing the test solution, add 1.5 mL of strong hydrogen peroxide after adding 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 50), then ignite (not more than 1 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Cefazolin Sodium in 20 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution by the automatic integration method and calculate the amount of related substances by the area percentage method: the amount of the related substance having the relative retention time of about 0.2 with respect to cefazolin and the amount of each related substance other than cefazolin is not more than 1.5 %, and the total area of the peaks other than cefazolin is not more than 2.5 %. The area of the peak having the relative retention time of about 0.2 with respect to the retention time of cefazolin is used after multiplying by its relative response factor, 1.43.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Detection sensitivity: Dissolve about 80 mg of Cefazolin RS in 0.1 mol/L phosphate buffer solution (pH 7.0) to make 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of this solution, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μL of this solution is equivalent to 3 to 7 % of that of cefazolin obtained from the system suitability solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0 %.

Time span of measurement: About 3 times as long as the retention time of cefazolin beginning after the solvent peak.

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cefazolin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS, add 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Cefazolin Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 2.5 % (1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination.

Sterility Test It meets the requirement when tested when Cefazolin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefazolin, when Cefazolin Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Cefazolin Sodium and Cefazolin RS, dissolve each in a suitable amount of the internal standard solution to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefazolin to that of the internal standard.

$$\text{Amount [μg (potency)] of cefazolin } (C_{14}H_{14}N_8O_4S_3) = \text{Amount [μg (potency)] of cefazolin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *p*-acetoanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefazolin and *p*-acetoanisidide are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefazolin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefazolin Sodium for Injection

Cefazolin Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefazolin Sodium for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Method of Preparation Prepare as directed under Injections, with Cefazolin Sodium

Description Cefazolin Sodium for Injection appears as white to yellowish white crystals, crystalline powder, or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium for Injection (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 270 nm and 274 nm.

(2) Cefazolin Sodium for Injection responds to the Qualitative Tests (1) for sodium salt.

pH The pH of a solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of cefazolin, in 10 mL of water is 4.5 to 6.5.

Purity (1) ***Clarity of solution***—Conduct this procedure within 10 minutes after the preparation of the solutions. A solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of cefazolin sodium according to the labeled amount, in 10 mL of water is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.35.

(2) ***Related substances***—Dissolve an amount of Cefazolin Sodium for Injection, equivalent to 0.10 g (potency) of cefazolin sodium according to the labeled amount, in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the test solution. Prepare the test solution before use. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the area percentage of each peak by the automatic integration method. Each area of the peaks other than cefazolin is not more than 1.5 %, and the total area of the peaks other than cefazolin is not more than 2.5 %. For these calculations, use the area of the peak, having the relative retention ime of about 0.2 with respect to cefazolin, after multiplying by the relative response factor, 1.43.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the assay under Cefazolin Sodium.

System suitability

Test for required detectability: Pipet 8 mL of the test solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the system suitability solution. Pipet 1 mL of this solution and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Confirm that the peak area of cefazolin sodium obtained from 5 μL of this solution is equivalent to 3 to 7 % of that from 5 μL of the system suitability solution.

System performance: Proceed as directed in the system suitability in the Assay under Cefazolin Sodium

System repeatability: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0 %.

Time span of measurement: About 3 times as long as the retention time of cefazolin beginning after the solvent peak.

Water Not more than 3.0 % (0.5 g, volumetric titration, direct titration). Use a mixture of formamide for the Karl Fischer method and methanol for the Karl Fischer method (2 : 1) instead of methanol for the Karl Fisher method.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.05 EU/mg (potency) of cefazolin sodium.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

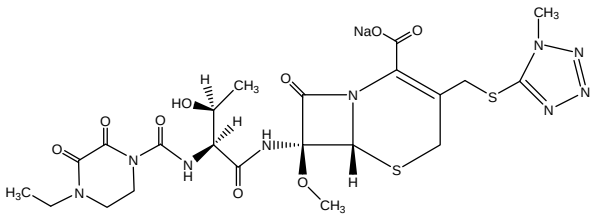
Assay Weigh accurately the mass of the contents of not less than 10 containers of Cefazolin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency), dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately 50 mg (potency) of Cefazolin RS, dissolve in the internal standard solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefazolin Sodium.

$$\text{Amount [μg (potency)] of cefazolin } (C_{14}H_{14}N_8O_4S_3) = \text{Amount [μg (potency)] of Cefazolin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20000)

Containers and Storage ***Containers***—Hermetic containers.

Cefbuperazone Sodium



$C_{22}H_{28}N_9NaO_9S_2$: 649.63

Sodium (6*R*,7*S*)-7-[[(2*S*,3*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amido]-3-hydroxybutanamido]-7-methoxy-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*76648-01-6*]

Cefbuperazone Sodium contains not less than 870 μg (potency) per mg of cefbuperazone ($C_{22}H_{29}N_9O_9S_2$: 627.65), calculated on the anhydrous basis.

Description Cefbuperazone Sodium appears as white to pale yellowish white powder or masses.
Cefbuperazone Sodium is very soluble in water, freely soluble in methanol or in pyridine, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Cefbuperazone Sodium and Cefbuperazone Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g of Cefbuperazone Sodium in 0.5 mL of deuterated pyridine for nuclear magnetic resonance spectroscopy and 1 drop of heavy water for nuclear magnetic resonance spectroscopy, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal, A, at around δ 1.1 ppm, and double signals, B and C, at around δ 1.6 ppm and at around δ 5.1 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C, is about 3 : 3 : 1.

(3) Cefbuperazone Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +48 ~ +56° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g (potency) of Cefbuperazone Sodium in 4 mL of water is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the solution is clear and pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Cefbuperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefbuperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Cefbuperazone Sodium in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the percentages of each peak area of the related substances from the test solution against 50 times of the peak area of cefbuperazone from the standard solution: the amount of the related substance having the relative retention time of about 0.2 with respect to cefbuperazone is not more than 2.0 %, the amount of the related substance having the relative retention time of about 0.6 with respect to cefbuperazone is not more than 4.5 %, the amount of the related substance having the relative retention time of about 1.6 with respect to cefbuperazone is not more than 1.0 %, and the total area of related substances is not more than 6.0 %. Use the peak areas of the related substances having the relative retention times of about 0.2 and 1.6 after multiplying by their relative response factors, 0.72 and 0.69, respectively.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefbuperazone obtained from 25 μL of this solution is equivalent to 7 to 13 % of that of cefbuperazone from the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of cefbuperazone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefbuperazone is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of cefbuperazone.

Water Not more than 1.0 % (3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cefbuperazone Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.1 EU/mg (potency) of cefbuperazone, when Cefbuperazone Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Cefbuperazone Sodium and Cefbuperazone RS, and dissolve each in the mobile phase to make exactly 100 mL. To 10 mL each of these solutions add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefbuperazone to that of the internal standard.

$$\text{Amount [μg (potency)] of cefbuperazone } (C_{22}H_{29}N_9O_9S_2) = \text{Amount [μg (potency)] of Cefbuperazone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 4000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 2.0 g of tetra-*n*-propylammonium bromide in 1000 mL of a mixture of water, acetonitrile, and acetic acid (pH 5.0)-sodium acetate buffer solution (83 : 13 : 4).

Flow rate: Adjust the flow rate so that the retention time of cefbuperazone is about 16 minutes.

System suitability

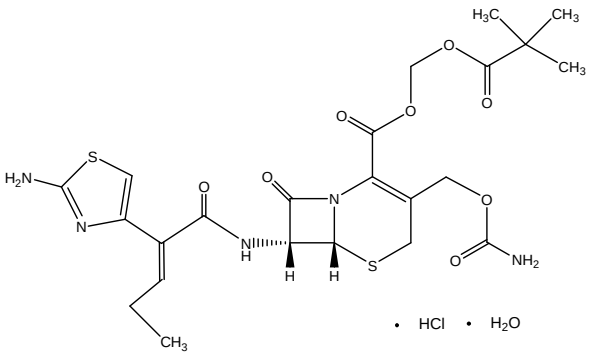
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefbuperazone are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefbuperazone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In a cold place.

Cefcapene Pivoxil Hydrochloride Hydrate



Cefcapene Pivoxil Hydrochloride
$C_{23}H_{29}N_5O_8S_2 \cdot HCl \cdot H_2O$: 622.11

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(2*E*)-2-(2-amino-1,3-thiazol-4-yl)pent-2-enamido]-3-(carbamoyloxymethyl)-3,4-didehydrocepham-4-carboxylate hydrate hydrochloride [*147816-24-8*]

Cefcapene Pivoxil Hydrochloride Hydrate contains not less than 722 μg (potency) and not more than 764 μg (potency) per mg of cefcapene ($C_{17}H_{19}N_5O_6S_2$: 453.49).

Description Cefcapene Pivoxil Hydrochloride Hydrate appears as white to pale yellowish white crystalline powder or masses having a slight characteristic odor.
Cefcapene Pivoxil Hydrochloride Hydrate is freely soluble in *N,N*-dimethylformamide or in methanol, soluble in ethanol (99.5), slightly soluble in water, and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in deuterated methanol for nuclear magnetic resonance spectroscopy (1 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal, A, at around δ 6.3 ppm and a single signal, B, at around δ 6.7 ppm. The ratio of the integrated intensity of each signal, A : B, is about 1 : 1.

(3) Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate in 2 mL of a mixture of water and methanol (1 : 1), and add 1 drop of silver nitrate TS: a white precipitate is formed.

Specific Optical Rotation $[\alpha]_D^{20}$: +51 ~ +54° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm)

Absorbance $E_{1cm}^{1\%}$ (265 nm): 255 ~ 285 (30 mg calculated on the anhydrous basis, a mixture of acetate buffer solution (pH 5.5) and methanol (1 : 1), 2000 mL).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefcapene Pivoxil Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substance I***—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 10 mg (potency), in 2 mL of methanol, add a mixture of water and methanol (1 : 1) to make 50 mL, and use this solution as the test solution. Perform the test with 30 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. If necessary, compensate for changes in the baseline by proceeding in the same manner with 30 μL of a mixture of water and methanol (1 : 1). Measure the amount of the peaks other than cefcapene pivoxil by the area percentage method: the amounts of the peaks having the relative retention times of about 1.5 and about 1.7 with respect to the peak of cefcapene pivoxil are not more than 0.2 %, respectively. The amount of the each peak other than the peaks mentioned above is not more than 0.1 %, and the total of them is not more than 1.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 5.99 g of potassium dihydrogen phosphate in water to make 1100 mL. To this solution add a solution obtained by dissolving 1.89 g of tetra-*n*-pentylammonium bromide in methanol to make 1000 mL.

Mobile phase B: A mixture of methanol and water (22 : 3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20	98	2

20-40	98→50	2→50
40-50	50	50

Flow rate: 0.8 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the tst solution, add a mixture of water and methanol (1 : 1) to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add a mixture of water and methanol (1 : 1) to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 30 μL of this solution is equivalent to 7 to 13 % of that of cefcapene pivoxil from the system suitability solution.

System performance: Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate and 10 mg of propyl parahydroxybenzoate in 25 mL of methanol, and add water to make 50 mL. To 5 mL of this solution add a mixture of water and methanol (1 : 1) to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, cefcapene pivoxil and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 30 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of cefcapene pivoxil is not more than 4.0 %.

Time span of measurement: About 2.5 times as long as the retention time of cefcapene pivoxil

(3) ***Related substance II***—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 2 mg (potency), in *N,N*-dimethylformamide for liquid chromatography to make 20 mL, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the test solution by the automatic integration method: the total area of the peaks that appear earlier than cefcapene pivoxil is not more than 1.7 % of the total area of the peaks other than the solvent.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 7.8 mm in internal diameter and about 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography.

Column temperature: A constant temperature of about 25 °C

Mobile phase: A solution of lithium bromide in *N,N*-dimethylformamide for liquid chromatography (13 in 5000)

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 22 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add *N,N*-dimethylformamide for liquid chromatography to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 3 mL of the system suitability solution, and add *N,N*-dimethylformamide for liquid chromatography to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 20 μL of this solution is equivalent to 20 to 40 % of that of cefcapene pivoxil from the system suitability solution.

System performance: When the procedure is run with 20 μL of the test solution under the above operating conditions, the number of theoretical plates of the peak of cefcapene pivoxil is not less than 12000.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of cefcapene pivoxil is not more than 4.0 %.

Time span of measurement: About 1.8 times as long as the retention time of cefcapene pivoxil.

Water 2.8 ~ 3.7 % (0.5 g, volumetric titration, back titration)

Assay Weigh accurately about 20 mg (potency) each of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS, and dissolve each in a mixture of water and methanol (1 : 1) to make exactly 50 mL. To 10 mL each of these solutions add exactly 10 mL of the internal standard solution and a mixture of water and methanol (1 : 1) to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefcapene pivoxil to that of the internal standard.

$$\text{Amount [μg (potency)] of cefcapene } (C_{17}H_{19}N_5O_6S_2) = \text{Amount [μg (potency)] of Cefcapene RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *p*-benzylphenol in a mixture of water and methanol (1 : 1) (7 in 4000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm)

Column: A stainless steel column about 3.0 mm in internal diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate and 1.22 g of sodium 1-decanesulfonate in water to make 1000 mL. To 700

mL of this solution add 300 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 5 minutes.

System suitability

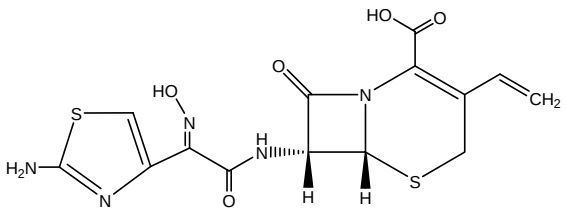
System performance: Dissolve 0.2 g of Cefcapene Pivoxil Hydrochloride Hydrate in 10 mL of methanol, and warm in a water bath at 60 °C for 20 minutes. After cooling, pipet 1 mL of this solution, and add exactly 10 mL of the internal standard solution and a mixture of water and methanol (1 : 1) to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cefcapene pivoxil, *trans*-cefcapene pivoxil, and the internal standard are eluted in this order, the ratios of the retention time of *trans*-cefcapene pivoxil and the internal standard to that of cefcapene pivoxil are 1.7 and 2.0, respectively, and the resolution between the peaks of *trans*-cefcapene pivoxil and the internal standard is not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefcapene pivoxil to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5 °C.

Cefdinir



$C_{14}H_{13}N_5O_5S_2$: 395.41

(6*R*,7*R*)-7-[(2*E*)-2-(2-Amino-1,3-thiazol-4-yl)-2-methoxyiminoacetamido]-3-ethenyl-3,4-didehydrocepham-4-carboxylic acid [*91832-40-5*]

Cefdinir contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg of cefdinir ($C_{14}H_{13}N_5O_5S_2$: 395.41).

Description Cefdinir appears as white to pale yellow crystalline powder.

Cefdinir is sparingly soluble in water, in ethanol (95), or in ether.

Cefdinir dissolves in 0.1 mol/L phosphate buffer solution (pH 7.0).

Identification (1) Determine the absorption spectra of solutions of Cefdinir and Cefdinir RS in 0.1 mol/L phosphate buffer solution (pH 7.0) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefdinir and Cefdinir RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefdinir in a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4 : 1) (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits multiple signals, A and B, between δ 5.0 and 6.1 ppm and between δ 6.4 and 7.5 ppm, respectively. The ratio of the integrated intensity of each signal, A : B, is about 2 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: -58 ~ -66° (0.25 g, 0.1 mol/L phosphate buffer solution (pH 7.0), 25 mL, 100 mm).

Absorbance $E_{1cm}^{1\%}$ (287 nm): 570 ~ 610 (50 mg, 0.1 mol/L phosphate buffer solution (pH 7.0), 5 L).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefdinir according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve about 0.1 g of Cefdinir in 10 mL of 0.1 mol/L phosphate buffer solution (pH 7.0). Pipet 3 mL of this solution, add tetramethylammonium hydroxide TS (pH 5.5) to make exactly 20 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of the test solution by the automatic integration method, and calculate the amounts by the area percentage method: the peak area of *E*-isomer having the relative retention time of 1.5 with respect to the peak of cefdinir is not more than 0.8 %, and the total area of the peaks other than cefdinir is not more than 3.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Flowing of the mobile phase: Control the step or concentration gradient by mixing mobile phases A and

B as directed in the following table.

Mobile phase A: To 1000 mL of tetramethylammonium hydroxide TS (pH 5.5) add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Mobile phase B: To 500 mL of tetramethylammonium hydroxide TS (pH 5.5) add 300 mL of acetonitrile for liquid chromatography and 200 mL of methanol, and add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-2	95	5
2-22	95→75	5→25
22-32	75→50	25→50
32-37	50	50
37-38	50→95	50→5
38-58	95	5

Flow rate: 1.0 mL/minute. The retention time of cefdinir is about 22 minutes under this condition.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add tetramethylammonium hydroxide TS (pH 5.5) to make exactly 100 mL, and use this solution as the system suitability solution. To 1 mL of the system suitability solution add tetramethylammonium hydroxide TS (pH 5.5) to make exactly 10 mL. Confirm that the peak area of cefdinir obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.

System performance: Dissolve 0.03 g of Cefdinir RS and 2 mg of cefdinir lactam ring-cleavage lactones in 3 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and add tetramethylammonium hydroxide TS (pH 5.5) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into four peaks, cefdinir, and peak 3 and peak 4 of cefdinir lactam ring-cleavage lactones are eluted in this order. The relative retention time of peak 3 of cefdinir lactam ring-cleavage lactone to the retention time of cefdinir is not less than 1.09. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 7000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 3 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 2.0 %.

Time span of measurement: 40 minutes after injection of the test solution

Water Not more than 2.0 % (1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination).

Assay Weigh accurately about 20 mg (potency) each of Cefdinir and Cefdinir RS, dissolve each in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with exactly 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefdinir.

$$\text{Amount [μg (potency)] of cefdinir } (C_{14}H_{13}N_5O_5S_2) = \text{Amount [μg (potency)] of Cefdinir RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: To 1000 mL of tetramethylammonium hydroxide TS (pH 5.5) add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS. To 900 mL of this solution add 60 mL of acetonitrile for liquid chromatography and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefdinir is about 8 minutes.

System suitability

System performance: Dissolve 2 mg of Cefdinir RS and 5 mg of cefdinir lactam ring-cleavage lactones in 10 mL of 0.1 mol/L phosphate buffer solution (pH 7.0). When the procedure is run with 5 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into four peaks, cefdinir, and peak 3 and peak 4 of cefdinir lactam ring-cleavage lactones are eluted in this order. The resolution between peak 2 of cefdinir lactam ring-cleavage lactone to that of cefdinir is not less than 1.2. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefdinir Capsules

Cefdinir Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$: 395.41).

Method of Preparation Prepare as directed under Capsules, with Cefdinir.

Identification To an amount of the contents of Cefdinir Capsules, equivalent to 10 mg (potency) of cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), sonicate for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Dissolution Test Perform the test with 1 capsule of Cefdinir Capsules at 50 revolutions per minute according to Method 2 under Dissolution Test using the sinker, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take not less than 20 mL of the dissolved solution 45 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 56 μg (potency) of cefdinir, and use this solution as the test solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir RS, and dissolve in the 2nd fluid for dissolution test to make exactly 100 mL. Pipet 4 mL of this solution, add the 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefdinir. The dissolution rate of Cefdinir Capsules in 45 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
= Amount [mg (potency)] of Cefdinir RS
$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

C: Labeled amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in 1 capsule

Operating conditions

Proceed as directed in the operating conditions in the Assay under Cefdinir.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 20 Cefdinir Capsules. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of cefdinir according to the labeled potency, add 70 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Centrifuge this solution at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the clear supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefdinir RS, dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefdinir.

Amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
$$= \text{Amount [mg (potency)] of Cefdinir RS} \times \frac{A_T}{A_S} \times 5$$

Containers and Storage ***Containers***—Tight containers.

Cefdinir Fine Granules

Cefdinir Fine Granules contain not less than 93.0 % and not more than 107.0 % of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$: 395.41).

Method of Preparation Prepare into fine granules as directed under Powders, with Cefdinir.

Identification Weigh an amount of Cefdinir Fine Granules, equivalent to 10 mg (potency) of cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), sonicate for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Particle Size Distribution Test It meets the requirement.

Dissolution Test Weigh accurately an amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of cefdinir according to the labeled amount, and perform the test at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take not less than 20 mL of the dissolved solution after 30 minutes from the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately an amount of Cefdinir RS, equivalent to about 28 mg (potency), and dissolve in the 2nd fluid for dissolution test to make exactly 50 mL. Pipet 4 mL of this solution, add the 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefdinir. The dissolution rate of Cefdinir Fine Granules in 30 minutes is not less than 75 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cefdinir } (C_{14}H_{13}N_5O_5S_2) = \frac{\text{Amount [mg (potency)] of Cefdinir RS}}{W_T} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 360$$

W_T: Amount (g) of Cefdinir Fine Granules taken
C: Labeled amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in 1 g

Operating conditions
Proceed as directed in the operating conditions in the Assay under Cefdinir.
System suitability
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0 %.

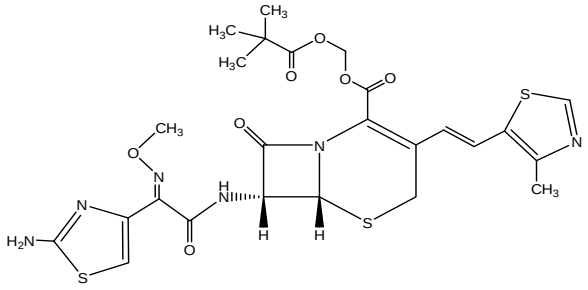
Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefdinir. Powder, if necessary, and weigh accurately an amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency), add 70 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution to make exactly 100 mL. Centrifuge at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefdinir RS, dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution.

$$\text{Amount [mg (potency)] of cefdinir } (C_{14}H_{13}N_5O_5S_2) = \text{Amount [mg (potency)] of Cefdinir RS} \times \frac{A_T}{A_S} \times 5$$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Cefditoren Pivoxil



$C_{25}H_{28}N_6O_7S_3$: 620.72

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(2*E*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetamido]-3-[2-(4-methyl-1,3-thiazol-5-yl)ethen-1-yl]-3,4-didehydrocepham-4-carboxylate [*117467-28-4*]

Cefditoren Pivoxil contains not less than 770 μg (potency) and not more than 820 μg (potency) per mg of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58), calculated on the anhydrous basis.

Description Cefditoren Pivoxil appears as pale yellowish white to pale yellow crystalline powder.
Cefditoren Pivoxil is sparingly soluble in methanol, slightly soluble in acetonitrile or in ethanol (95), very slightly soluble in ether, and practically insoluble in water.
Cefditoren Pivoxil dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 5 mg of Cefditoren Pivoxil in 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.
(2) Dissolve 1 mg of Cefditoren Pivoxil in 1 mL of dilute hydrochloric acid and 4 mL of water, add 3 drops of sodium nitrite TS while cooling, shake, and allow to stand for 2 minutes. Then add 1 mL of ammonium amidosulfate TS, shake well, allow to stand for 1

minute, and add 1 mL of a solution obtained by dissolving 1.0 g of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate in 100 mL of a mixture of acetone and water (1 : 1): a purple color develops. Prepare the mixture of acetone and water (1 : 1) before use.

(3) Determine the absorption spectra of solutions of Cefditoren Pivoxil and Cefditoren Pivoxil RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the ^{1}H spectrum of a solution of Cefditoren Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B, and C at around δ 1.1 ppm, at around δ 2.4 ppm, and at around δ 4.0 ppm, double signals D and E at around δ 6.4 ppm and at around δ 6.7 ppm, and a single signal F at around δ 8.6 ppm. The ratio of the integrated intensity of each signal, A : B : C : D : E : F, is about 9 : 3 : 3 : 1 : 1 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: -45 ~ -52° (50 mg, methanol, 10 mL, 100 mm).

Absorbance $E_{1cm}^{1\%}$ (231 nm): 340 ~ 360 (50 mg, methanol, 2500 mL).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefditoren Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Conduct this procedure using light-resistant vessels. Weigh accurately about 20 mg (potency) of Cefditoren Pivoxil, add exactly 5 mL of the internal standard solution and acetonitrile to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil RS, and add acetonitrile to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 5 mL of the internal standard solution and acetonitrile to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_{TI}, Q_{TII}, and Q_{TII}, of the peak areas of related substances I, II, and III to the peak area of the internal standard in the test solution, and the ratio, Q_S, of the peak area of cefditoren pivoxil to that of the internal standard in the standard solution. (Related substance I is not more than 1.5 %, related substance II is not more than 2.0 %, and related substance III is not more than 1.0 %.) Use the peak areas of related substance I, II, and III after multiplying the areas obtained according to the automatic integration method by their relative response factors, 1.25, 0.97, and 1.17, respectively.

$$\text{Content (\%) of related substances} = \frac{Q_T}{Q_S} \times \frac{W_S}{W_T}$$

W_S: Amount (mg) of Cefditoren Pivoxil RS taken

$$\times \frac{\text{Amount } [\mu\text{g/mg (potency)}] \text{ of Cefditoren Pivoxil RS}}{816}$$

W_T: Amount (mg) of Cefditoren Pivoxil taken

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in acetonitrile (1 in 40000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 250 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 1.58 g of ammonium formate in 900 mL of water, adjust the pH to about 6.0 with diluted formic acid (1 in 250), and add water to make exactly 1000 mL. To 450 mL of this solution add 275 mL of acetonitrile and 275 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefditoren pivoxil is about 15 minutes. Related substances I, II, and III are eluted at about 6 minutes, at about 17 minutes, and at about 22 minutes, respectively, under this condition.

Selection of column: Dissolve 20 mg of Cefditoren Pivoxil RS and 5 mg of propyl *p*-hydroxybenzoate in 20 mL of acetonitrile, and proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of propyl *p*-hydroxybenzoate and cefditoren pivoxil in this order with the resolution between these peaks being not less than 5.0.

(3) ***Other related substances***—Conduct this procedure using light-resistant vessels. Weigh accurately 20 mg (potency) of Cefditoren Pivoxil, add acetonitrile to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil RS, and dissolve in acetonitrile to make exactly 100 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution under the conditions of (2) except detection sensitivity and time span of measurement: each peak area other than cefditoren pivoxil and other than related substances I, II, and III from the test solution is not larger than the peak area of cefditoren pivoxil from the standard solution (not more than 1.0 %), and the sum area of the peaks is not larger than 2 times the peak area of cefditoren pivoxil from the standard solution (not more than 2.0 %).

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cefditoren pivoxil obtained from 10 μL of the standard solution is equivalent to 5 to 10 % of the full scale.

Time span of measurement: About 2 times as long as the retention time of cefditoren pivoxil beginning after the solvent peak.

Water Not more than 1.5 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g)

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 40 mg (potency) each of Cefditoren Pivoxil and Cefditoren Pivoxil RS, dissolve each in 40 mL of acetonitrile, add exactly 10 mL of the internal standard solution and acetonitrile to make 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefditoren pivoxil to that of the internal standard.

$$\text{Amount [μg (potency)] of Cefditoren } (C_{19}H_{18}N_6O_5S_3) = \text{Amount [μg (potency)] of Cefditoren Pivoxil RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in acetonitrile (1 in 200)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 1.58 g of ammonium formate in 900 mL of water, adjust the pH to about 6.0 with diluted formic acid (1 in 250), and add water to make exactly 1000 mL. To 450 mL of this solution add 275 mL of acetonitrile and 275 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefditoren pivoxil is about 15 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefditoren pivoxil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution, the relative standard deviation of the ratios of the peak area of cefditoren pivoxil to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefditoren Pivoxil Fine Granules

Cefditoren Pivoxil Granules

Cefditoren Pivoxil Fine Granules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58).

Method of Preparation Prepare into fine granules as directed under Powders, with Cefditoren Pivoxil.

Identification (1) To an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to 0.1 g (potency) of cefditoren pivoxil according to the labeled amount, add 10 mL of acetonitrile, shake well, and filter. To 1 mL of the filtrate add acetonitrile to make 50 mL. To 1 mL of this solution add acetonitrile to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 230 nm and 234 nm.

(2) Proceed as directed in the Assay: the test solution and standard solution exhibit peaks at the same retention time.

Purity (1) ***Related substances***—Perform the test as directed in the Purity (2) under Cefditoren Pivoxil. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 16 mg (potency) according to the labeled potency, add 10 mL of diluted acetonitrile (3 in 4), shake, add exactly 5 mL of the internal standard solution and diluted acetonitrile to make exactly 20 mL, and filter. Centrifuge the filtrate and use as the test solution (related substance I is not more than 2.0 %, related substance II is not more than 3.5 %, and related substance III is not more than 1.0 %).

(2) ***Other related substances***—Perform the test as directed in the Purity (3) under Cefditoren Pivoxil. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 16 mg (potency) according to the labeled potency, add 10 mL of diluted acetonitrile (3 in 4), shake, add diluated acetonitrile (3 in 4) to make exactly 20 mL, filter, centrifuge, and use as the test solution. The area of each peak other than cefditoren pivoxil and related substances I, II and III from the test solution is not larger than the peak area of cefditoren pivoxil from the standard solution (not more than 1.0 %), and the total area of the peaks is not larger than 2 times the peak area of cefditoren pivoxil from the standard solution (not more than 2.0 %).

Loss on Drying Not more than 4.5 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test as directed in the Dissolution Test under Cefditoren Pivoxil Tablets. Weigh accurately an amount of Cefditoren Pivoxil Fine Granules, equivalent to about 0.1 g (potency) according to the labeled amount, and perform the test at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of the 1st fluid for dissolution test as the dissolution solution. Take not less than 20 mL of the dissolved solution after 20 minutes from the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the test solution. Determine the absorbances at 272 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Cefditoren Pivoxil Fine Granules in 20 minutes is not less than 80 % of the labeled amount.

Particle Size Distribution Test It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 40 mg (potency) of cefditoren pivoxil according to the labeled amount, add 70 mL of diluted acetonitrile (3 in 4), and shake vigorously. To this solution add exactly 10 mL of the internal standard solution and acetonitrile to make 100 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil RS, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefditoren Pivoxil.

Amount [μg (potency)] of Cefditoren ($C_{19}H_{18}N_6O_5S_3$)
= Amount [μg (potency)] of Cefditoren Pivoxil RS

$$\times \frac{Q_T}{Q_S} \times 2$$

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200)

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefditoren Pivoxil Tablets

Cefditoren Pivoxil Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58).

Method of Preparation Prepare as directed under Tablets, with Cefditoren Pivoxil.

Identification (1) To an amount of powdered Cefditoren Pivoxil Tablets, equivalent to 35 mg (potency) of cefditoren pivoxil according to the labeled amount, add 100 mL of methanol, shake, and filter. To 5 mL of the filtrate add methanol to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 229 nm and 233 nm.

(2) Proceed as directed in the Assay: the test solution and standard solution exhibit peaks at the same retention time.

Loss on Drying Not more than 4.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 tablet of Cefditoren Pivoxil Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of the first solution under Dissolution Test as the dissolution solution. Take not less than 20 mL of the dissolved solution 20 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg (potency) of cefditoren pivoxil, and use this solution as the test solution. Separately, weigh accurately about 22 mg (potency) of Cefditoren Pivoxil RS, dissolve in 20 mL of diluted acetonitrile (3 in 4), and add the first solution under Dissolution Test to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 272 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. The dissolution rate of Cefditoren Pivoxil Tablets in 20 minutes is not less than 85 %.

Dissolution rate (%) with respect to the labeled amount of cefditoren pivoxil ($C_{19}H_{18}N_6O_5S_3$)
= Amount [mg (potency)] of Cefditoren Pivoxil RS

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 45$$

C: Labeled amount [mg (potency)] of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$) in 1 tablet

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.
Conduct this procedure using light-resistant vessels. To 1 tablet of Cefditoren Pivoxil Tablets add exactly 12.5 mL of the 1st fluid for disintegration test, and shake vigorously. Add 25 mL of acetonitrile, shake, and add acetonitrile to make exactly 50 mL. Pipet *V* mL, equivalent to about 20 mg (potency) of cefditoren pivoxil according to the labeled amount, add exactly 5 mL of the internal standard solution and diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefditoren Pivoxil.

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200)

Assay Conduct this procedure using light-resistant vessels. To an amount of Cefditoren Pivoxil Tablets, equivalent to 0.5 g (potency) of cefditoren pivoxil according to the labeled amount, add 63 mL of the first solution under Disintegration Test, shake vigorously, add 125 mL of acetonitrile, shake, and add acetonitrile to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil RS, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefditoren Pivoxil.

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200)

Containers and Storage ***Containers***—Tight containers.

Cefepime Dihydrochloride for Injection

Cefepime Dihydrochloride for Injection is a preparation for injection, which is dissolved before use.
Cefepime Dihydrochloride for Injection contains not less than 95.0 % and not more than 110.0 % of the labeled amount of cefepime ($C_{19}H_{24}N_6O_5S_2$: 480.56).

Method of Preparation Prepare as directed under Injections, with Cefepime Dihydrochloride Hydrate.

Description Cefepime Dihydrochloride for Injection appears as white to pale yellow powder.

Identification Perform the test with Cefepime Dihydrochloride for Injection as directed in the Identification (1) and (3) under Cefepime Dihydrochloride Hydrate.

pH The pH of a solution obtained by dissolving an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.1 g (potency) of cefepime dihydrochloride, in 1 mL of water is between 4.0 and 6.0.

Purity (1) ***Color of solution***—Dissolve an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of cefepime dihydrochloride hydrate according to the labeled amount, in 5 mL of water: the solution is colorless to pale yellow.
(2) ***N-Methylpyrrolidine***—Perform the test as directed in Purity (2) under Cefepime Dihydrochloride Hydrate. Weigh accurately about 0.2 g (potency) of Cefepime Dihydrochloride for Injection, according to the labeled potency, dissolve in diluted nitric acid (2 in 625) to make exactly 20 mL, and use this solution as the test solution (not more than 1.0 %).

Water Not more than 4.0 %. Perform the test as directed in Water under Cefepime Dihydrochloride hydrate (volumetric titration, direct titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins It meets the requirement. Less than 0.06 EU/mg (potency) of cefepime. Dissolve about 1 g (potency) of Cefepime Dihydrochloride for Injection, according to the labeled potency, in water for bacterial endotoxins test. If necessary, adjust the pH to 6.0 to 7.5 with 0.1 mol/L sodium hydroxide TS for bacterial endotoxins test or 0.1 mol/L hydrochloric acid TS for bacterial endotoxins test, then add water for bacterial endotoxins test to make a solution so that each mL contains about 0.1 g (potency). Pipet an amount of this solution, and add water for bacterial endotoxins test to make a test solution of suitable concentration.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

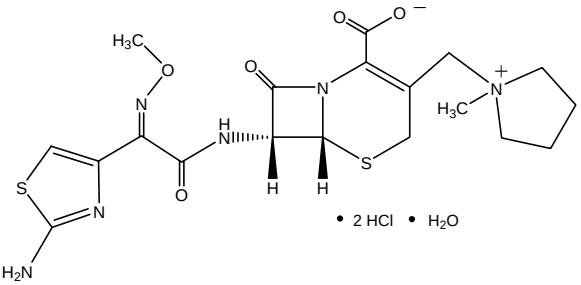
Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefepime Dihydrochloride Hydrate. Weigh accurately about 60 mg (potency) of Cefepime Dihydrochloride for Injection, according to the labeled potency, dissolve

in the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefepime Dihydrochloride Hydrate



$C_{19}H_{24}N_6O_5S_2 \cdot 2HCl \cdot H_2O$: 571.50

(6*R*,7*R*)-7-{2-[(2*Z*)-2-(2-Amino-1,3-thiazol-4-yl)-2-methoxyimino]acetamido}-3-(1-methylpyrrolidin-1-ium-1-yl)methyl-3,4-didehydrocepham-4-carboxylate hydrate dihydrochloride [*123171-59-5*]

Cefepime Dihydrochloride Hydrate contains not less than 835 μg (potency) and not more than 886 μg (potency) per mg of cefepime ($C_{19}H_{24}N_6O_5S_2$: 480.56), calculated on the anhydrous basis.

Description Cefepime Dihydrochloride Hydrate appears as white to yellowish white crystals or crystalline powder.
Cefepime Dihydrochloride Hydrate is freely soluble in water or in methanol, slightly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) Dissolve 20 mg (potency) of Cefepime Dihydrochloride Hydrate in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Dissolve 15 mg (potency) of Cefepime Dihydrochloride Hydrate in 5 mL of water, and add 2 drops of silver nitrate TS: a white turbidity is produced.

(3) Determine the absorption spectra of solutions of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) Dissolve 50 mg (potency) of Cefepime Dihydrochloride Hydrate in 5 mL of heavy water, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals at around 3.1 ppm and around 7.2 ppm, and the ratio of integrated intensity of each signal is about 3 : 1.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +39 ~ +47° (60 mg calculated on the anhydrous basis, water, 20 mL, 100 mm)

Absorbance $E_{1cm}^{1\%}$ (259 nm): 310 ~ 340 (50 mg calculated on the anhydrous basis, water, 1 mL)

pH Dissolve 0.1 g (potency) of Cefepime Dihydrochloride Hydrate in 10 mL of water: the pH of this solution is between 1.6 and 2.1.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Cefepime Dihydrochloride Hydrate in 5 mL of a solution of L-arginine (3 in 50): the solution is clear and has no more color than Matching Fluid H.

(2) ***Heavy metals***—Proceed with about 1.0 g of Cefepime Dihydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances*** (i) *N*-Methylpyrrolidine—Weigh accurately about 80 mg (potency) of Cefepime Dihydrochloride Hydrate, dissolve in diluted nitric acid (2 in 3125) to make exactly 10 mL, and use this solution as the test solution. Separately, put 30 mL of water in a 100 mL volumetric flask, weigh accurately the mass of the flask, then add about 0.125 g of *N*-Methylpyrrolidine RS, weigh accurately the mass of the flask again, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of *N*-methylpyrrolidine in the test solution and standard solution (not more than 0.5 %).

$$\text{Amount (\%) of } N\text{-methylpyrrolidine} = \frac{\text{Amount (mg) of } N\text{-Methylpyrrolidine RS taken} \times f}{\text{Amount (mg) of Cefepime Dihydrochloride Hydrate taken}} \times \frac{A_T}{A_S} \times \frac{1}{250}$$

f: Purity (%) of *N*-methylpyrrolidine RS

Operating conditions

Detector: An electric conductivity detector

Column: A plastic tube about 4.6 mm in internal diameter and about 5 cm in length, packed with hydrophilic silica gel for liquid chromatography carrying sulfonic acid groups having the exchange capacity of about 0.3 meq/g (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: To 990 mL of diluted nitric acid (2 in 3125) add 10 mL of acetonitrile.

Flow rate: 1.0 mL/minute

System suitability

System performance: To 20 mL of a solution of sodium chloride (3 in 1000) add about 0.125 g of *N*-Methylpyrrolidine RS, and add water to make 100 mL. Pipet 4 mL of this solution, and add diluted nitric acid (2 in 3125) to make 100 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, sodium and *N*-methylpyrrolidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N*-methylpyrrolidine is not more than 4.0 %.

(ii) Other related subtances—Dissolve about 0.1 g (potency) of Cefepime Dihydrochloride Hydrate in the mobile phase A to make exactly 50 mL, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution, and calculate the amount of related substances by the area percentage method (not more than 0.5 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Increase the gradient of the mobile phase B in the mobile phase A from 0 % to 25 % in 25 minutes at the rate of 1 % per minute.

Mobile phase A: Dissolve 0.57 g of ammonium dihydrogen phosphate in 1000 mL of water.

Mobile phase B: Acetonitrile

Flow rate: Adjust the flow rate so that the retention time of cefepime is about 9.5 minutes.

System suitability

Test for required detectability: To 1.0 mL of the test solution add the mobile phase A to make 10 mL, and use this solution as the solution for system suitability test. To 1 mL of the solution for system suitability test add the mobile phase A to make 10 mL, and use this solution as the solution for test for required detectability. Pipet 1 mL of the solution for test for required detectability, and add the mobile phase A to make 10 mL. Confirm that the peak area of cefepime obtained from 5 μL of this solution is equivalent to 7 to 13 % of that of cefepime obtained from 5 μL of the solution for test for required detectability.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 6000.

System repeatability: When the test is repeated 3 times with 5 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of cefepime

Water 3.0 ~ 4.5 %. Weigh accurately about 50 mg of Cefepime Dihydrochloride Hydrate, and dissolve in 2 mL of methanol for water determinatio. Pipet 0.5 mL of this solution and perform the test (volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g)

Sterility Test It meets the requirement, when Cefepime Dihydrochloride Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.04 EU/mg (potency) of cefepime, when Cefepime Dihydrochloride Hydrate is used in a sterile preparation. Weigh accurately about 30 mg (potency) of Cefepime Dihydrochloride Hydrate, dissolve in water for bacterial endotoxins test, adjust the pH to 6.0 to 7.5 with 1 mol/L sodium hydroxide TS for bacterial endotoxins test or 1 mol/L hydrochloric acid TS for bacterial endotoxins test, and add water for bacterial endotoxins test to make exactly 5 mL. Pipet an amount of this solution, and add water for bacterial endotoxins test to make a test solution of suitable concentration.

Assay Weigh accurately about 60 mg (potency) each of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS, dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefepime in the test solution and standard solution.

Amount [μg (potency)] of cefepime ($C_{19}H_{24}N_6O_5S_2$)
= Amount [μg (potency)] of Cefepime Dihydrochloride

$$\text{RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Adjust a solution of sodium 1-pentanesulfonate (261 in 100000) to pH 3.4 with acetic acid (100), then adjust this solution to pH 4.0 with a solution of potassium hydroxide (13 in 20). To 950 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefepime is about 8 minutes.

System suitability

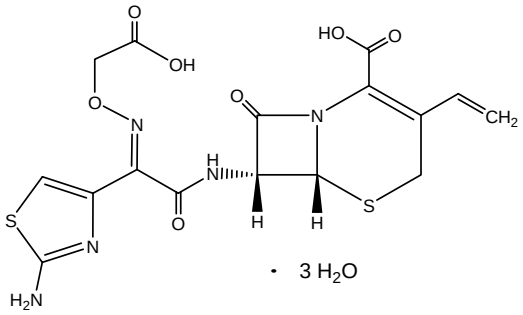
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 1500.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefixime Hydrate



$C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$: 507.50

(6*R*,7*R*)-7-[(2*E*)-2-(2-Amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetamido]-3-ethenyl-3,4-didehydrocepham-4-carboxylic acid trihydrate [*125110-14-7*]

Cefixime Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg of cefixime ($C_{16}H_{15}N_5O_7S_2$: 453.45), calculated on the anhydrous basis.

Description Cefixime Hydrate appears as white to pale yellow crystalline powder.

Cefixime Hydrate is freely soluble in methanol or in dimethylsulfoxide, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Cefixime Hydrate and Cefixime RS in 0.1 mol/L phosphate buffer solution (pH 7.0) (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefixime Hydrate and Cefixime RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Cefixime Hydrate in 0.5 mL of a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4 : 1), and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal, A, at around δ 4.7 ppm and a multiple signal, B, at around δ 6.5 ~ δ 7.4 ppm. The ratio of the integrated intensity of each signal, A : B, is about 1 : 1.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -75 ~ -88°(0.45 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 50), 50 mL, 100 mm).

pH The pH of a saturated solution of Cefixime Hydrate in water is between 2.4 and 4.1.

Absorbance $E_{1cm}^{1\%}$ (288 nm): 500 ~ 550 (70 mg calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution, pH 7.0, 5 L).

Purity ***Related substances***—Dissolve 0.1 g of Cefixime Hydrate in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, measure the each peak areas by the automatic integration method, and calculate the amounts of each peaks by the area percentage method: the amount of each peak other than cefixime is not more than 1.0 %, and the total amount of the peaks other than cefixime is not more than 2.5. %.

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Confirm that the peak height of cefixime obtained from 10 μL of this solution is 20 to 60 mm.

System performance: Dissolve about 2 mg of Cefixime RS in 200 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the system suitability solution. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of cefixime is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of the peak of cefixime

Water 9.0 ~ 12.0 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 0.1 g (potency) each of Cefixime Hydrate and Cefixime RS, and dissolve each in 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Pipet 10 mL each of these solutions, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefixime.

$$\text{Amount [μg (potency)] of cefixime } (C_{16}H_{15}N_5O_7S_2) = \text{Amount [μg (potency)] of Cefixime RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4 mm in internal diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter)

Column temperature: A constant temperature of about 40 °C

Mobile phase: To 25 mL of a solution of tetrabutylammonium hydroxide TS (10 in 13) add water to make 1000 mL, and adjust the pH to 6.5 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefixime is about 10 minutes.

System suitability

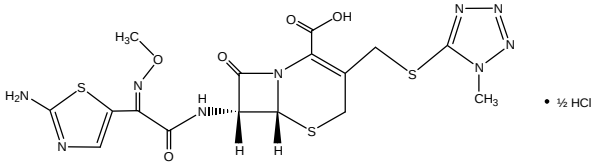
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefixime is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefmenoxime Hydrochloride



$C_{16}H_{17}N_9O_5S_3 \cdot ½HCl$: 529.79

(6*R*,7*R*)-7-[(2*E*)-2-(2-Amino-1,3-thiazol-5-yl)-2-methoxyiminoacetamido]-3-[2-(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylic acid hemihydrochloride [*75738-58-8*]

Cefmenoxime Hydrochloride contains not less than 890 μg (potency) and not more than 975 μg (potency) per mg of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$: 511.56), calculated on the anhydrous basis.

Description Cefmenoxime Hydrochloride appears as white to pale orange-yellow crystals or crystalline powder.

Cefmenoxime Hydrochloride is freely soluble in formamide or in dimethylsulfoxide, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution (pH 6.8) (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 230 nm and 234 nm and between 255 nm and 259 nm.

(2) Determine the infrared spectra of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotomtery: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg (potency) of Cefmenoxime Hydrochloride in 0.5 mL of deuterated dimethylsulfoxide, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits two single signals at around 3.9 ppm and

a single signal at around 6.8 ppm. The ratio of the integrated intensity of each signal is about 3 : 3 : 3.

(4) Dissolve 10 mg (potency) of Cefmenoxime Hydrochloride in 1 mL of diluted sodium carbonate TS (1 in 20), and add 5 mL of acetic acid (100) and 2 drops of silver nitrate TS: a white precipitate is formed.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -27 ~ -35° (1 g, 0.1 mol/L phosphate buffer solution (pH 6.8), 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 10 mg (potency) of Cefmenoxime Hydrochloride in 150 mL of water is between 2.8 and 3.3.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefmenoxime Hydrochloride in 10 mL of diluted sodium carbonate TS (1 in 4): the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. After cooling, add 10 mL of dilute hydrochloric acid to the residue (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution (pH 6.8), and add the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of 1-methyl-1*H*-tetrazol-5-thiol, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as standard solution (1). Separately, weigh accurately about 0.1 g of Cefmenoxime Hydrochloride RS, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution (pH 6.8), and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as standard solution (2). Perform the test immediately after preparation of these solutions with exactly 10 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution. Calculate the amount of 1-methyl-1*H*-tetrazol-5-thiol and the total amount of related substances by the following equations: not more than 1.0 % and not more than 3.0 %, respectively.

$$1-\text{methyl-}1H-\text{tetrazol-}5-\text{thiol}\,(\%)=\frac{W_{Sa}}{W_T}\times\frac{A_{Ta}}{A_{Sa}}\times 20$$

$$\text{Total amount (\%) of related substances}=\{\frac{W_{Sa}}{W_T}\times\frac{A_{Ta}}{A_{Sa}}\times 20\}+\{\frac{W_{Sb}}{W_T}\times\frac{S_T}{A_{Sb}}\times 5\}$$

W_{Sa}: Amount (g) of 1-methyl-1*H*-tetrazol-5-thiol

W_{Sb}: Amount (g) of Cefmenoxime Hydrochloride RS

W_T: Amount (g) of Cefmenoxime Hydrochloride

A_{Sa}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from standard solution (1)

A_{Sb}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from standard solution (2)

A_{Ta}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the test solution

S_T: Total area of the peaks other than 1-methyl-1*H*-tetrazol-5-thiol and cefmenoxime from the test solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of standard solution (1), and add the mobile phase to make exactly 100 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazol-5-thiol obtained from 10 μL of this solution is equivalent to 4.5 to 5.5 % of that of 1-methyl-1*H*-tetrazol-5-thiol from standard solution (1). Pipet 2 mL of standard solution (2), and add the mobile phase to make exactly 100 mL. Confirm that the peak area of cefmenoxime obtained from 10 μL of this solution is equivalent to 1.5 to 2.5 % of that of cefmenoxime from standard solution (2).

System repeatability: When the test is repeated 6 times with 10 μL each of standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0 %.

Time span of measurement: About 2.5 times as long as the retention time of cefmenoxime.

Water Not more than 1.5 % (1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol (2 : 1) instead of methanol for water determination.

Sterility Test It meets the requirement, when Cefmenoxime Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.083 EU/mg (potency) of cefmenoxime, when Cefmenoxime Hydrochloride is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride RS, dissolve each in 10 mL of 0.1

mol/L phosphate buffer solution (pH 6.8), and add the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefmenoxime to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefmenoxime } (C_{16}H_{17}N_9O_5S_3) = \text{Amount [μg (potency)] of Cefmenoxime RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of phthalimide in methanol (3 in 2000)

Operating conditions

Detector: An ultraviolet absorption photometer (254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 1000 mL of water add 200 mL of acetonitrile and 20 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of cefmenoxime is about 8 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmenoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.3.

System repeatability: When the test is repeated 6 tmies with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmenoxime to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefmenoxime Hydrochloride for Injection

Cefmenoxime Hydrochloride for Injection is a preparation for injection, which is dissolved before use.
Cefmenoxime Hydrochloride for Injection contains not less than 90.0 % and not more than 120 % of the labeled amount of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$: 511.56).

Method of Preparation Prepare as directed under Injections, with Cefmenoxime Hydrochloride.

Description Cefmenoxime Hydrochloride for Injection appears as white to pale orange-yellow powder.

Identification (1) Weigh an amount of Cefmenoxime Hydrochloride for Injection, equivalent to 10 mg (potency) of cefmenoxime, dissolve in 2 mL of 1 mol/L sodium hydroxide TS and 1 mL of a solution of hydroxylamine hydrochloride (1 in 10), allow to stand for 5 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a reddish purple color is produced.

(2) Weigh an amount of Cefmenoxime Hydrochloride for Injection, equivalent to 10 mg (potency) of cefmenoxime, dissolve in 2 mL of 1 mol/L sodium hydroxide TS, heat in a water bath for 1 minute. After cooling, add 1 drop of sodium nitroprusside TS: a red color is produced.

(3) Perform the test as directed in the Identification (1) under Cefmenoxime Hydrochloride.

pH The pH of a solution obtained by dissolving an amount of Cefmenoxime Hydrochloride for Injection, equivalent to 1.0 g (potency) of cefmenoxime, in 10 mL of water is between 6.4 and 7.9.

Loss on Drying Not more than 1.3 % (0.1 g, 0.7 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.083 EU/mg (potency) of cefmenoxime.

Foreign Insoluble Matter Test It meets the requirement.

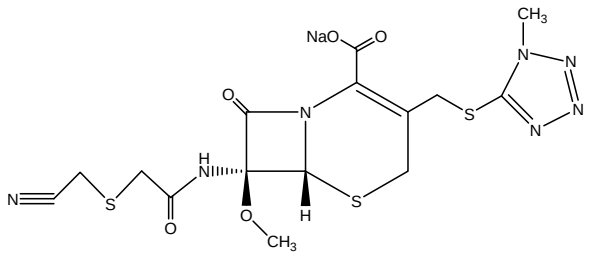
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefmenoxime Hydrochloride. Weigh accurately an amount of Cefmenoxime Hydrochloride for Injection, equivalent to about 0.5 g (potency) according to the labeled potency, and dissolve in water to make a solution so that each mL contains 5 mg (potency). Pipet 10 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefmetazole Sodium



$C_{15}H_{16}N_7NaO_5S_3$: 493.52

Sodium (6*R*,7*S*)-7-[2-(cyanomethylsulfanyl)acetamido]-7-methoxy-3-[2-(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*56796-39-5*]

Cefmetazole Sodium contains not less than 860 μg (potency) and not more than 965 μg (potency) per mg of cefmetazole ($C_{15}H_{17}N_7O_5S_3$: 471.53), calculated on the anhydrous basis.

Description Cefmetazole Sodium appears as white to pale yellowish white powder or masses.
Cefmetazole Sodium is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in tetrahydrofuran.
Cefmetazole Sodium is hygroscopic.

Identification (1) Dissolve 25 mg (potency) of Cefmetazole Sodium in water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 270 nm and 274 nm.

(2) Determine the infrared spectra of Cefmetazole Sodium and Cefmetazole Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefmetazole Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals at around δ 3.6 ppm, at around δ 4.1 ppm, and at around δ 5.2 ppm. The ratio of the integrated intensity of these signals is 3 : 3 : 1.

(4) Cefmetazole Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +73 ~ +85° (0.25 g, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1 g (potency) of Cefmetazole Sodium in 10 mL of water is between 4.2 and 6.2.

Absorbance $E_{1cm}^{1\%}$ (272 nm): 200 ~ 230 (25 mg calculated on the anhydrous basis, water, 1000 mL)

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefmetazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Cefmetazole Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve about 0.1 g of Cefmetazole Sodium in 2 mL of water, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 25 mL, and use this solution as standard solution (1). Separately, dissolve 0.10 g of 1-methyl-1*H*-tetrazol-5-thiol in water to make exactly 100 mL, and use this solution as standard solution (2). Immediately perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (4 : 1 : 1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot obtained from the test solution corresponding to the spot from standard solution (2) is not more intense than the spot from standard solution (2), and the spots other than this spot and other than the principal spot are not more intense than the spot from standard solution (1).

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefmetazole Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.06 EU/mg (potency) of cefmetazole, when Cefmetazole Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefmetazole Sodium and Cefmetazole RS, and dissolve each in the mobile phase to make exactly 25 mL. Pipet 1 mL each of these solutions, add exactly 10 mL of the internal standard solution and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefmetazole to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefmetazole } (C_{16}H_{17}N_7O_5S_3) = \text{Amount [μg (potency)] of Cefmetazole RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.75 g of ammonium dihydrogen phosphate in 700 mL of water. To this solution add 280 mL of methanol, 20 mL of tetrahydrofuran, and 3.2 mL of 40 % tetrabutylammonium hydroxide TS, and adjust the pH to 4.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cefmetazole is about 8 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmetazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeatead 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmetazole to that of the internal standard is 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Cefmetazole Sodium for Injection

Cefmetazole Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefmetazole Sodium for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of cefmetazole ($C_{15}H_{17}N_7O_5S_3$: 471.53).

Method of Preparation Prepare as directed under Injections, with Cefmetazole Sodium.

Description Cefmetazole Sodium for Injection appears as white to pale yellow powder or masses.
Cefmetazole Sodium for Injection is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Cefmetazole Sodium for Injection and Cefmetazole Sodium RS (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefmetazole Sodium for Injection and Cefmetazole Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH The pH of a solution obtained by dissolving an amount of Cefmetazole Sodium for Injection, equivalent to 1.0 g (potency) of cefmetazole sodium, in 10 mL of water is between 4.2 and 6.2.

Purity (1) ***Clarity and color of solution***—Dissolve an amount of Cefmetazole Sodium for Injection, equivalent to 1.0 g (potency) of cefmetazole sodium according to the labeled amount, in 10 mL of water: the solution is clear and the color is not darker than the following control solution.

Control solution: Pipet 0.5 mL of cobal (II) chloride hexahydrate stock CS and 5 mL of iron (III) chloride stock CS, and add water to make exactly 50 mL. Pipet 15 mL of this solution and add water to make exactly 20 mL.

(2) ***Related substances***—Perform the test as directed in the Purity (4) under Cefmetazole Sodium.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.06 EU/mg (potency) of cefmetazole.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

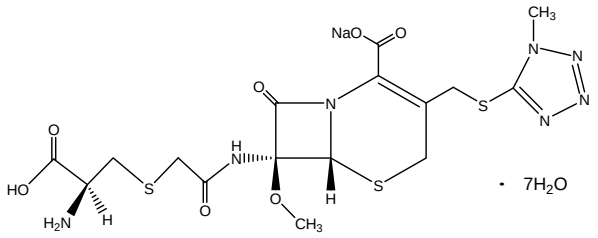
Assay Take not less than 10 containers of Cefmetazole Sodium for Injection, dissolve the contents of each in the mobile phase, combine the solutions, and add the mobile phase to make exactly 500 mL. Pipet a volume of this solution, equivalent to about 0.2 g (potency) of cefmetazole sodium, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the test solution. Separately, weigh accurately an amount of Cefmetazole Sodium RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefmetazole Sodium.

$$\text{Amount [μg (potency)] of cefmetazole } (C_{16}H_{17}N_7O_5S_3) = \text{Amount [μg (potency)] of Cefmetazole RS} \times \frac{Q_T}{Q_S} \times 4$$

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10000)

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefminox Sodium Hydrate



Cefmenox Sodium $C_{16}H_{20}N_7NaO_7S_3 \cdot 7H_2O$: 667.66

Sodium (6*R*,7*S*)-7-[2-[(2*S*)-2-amino-2-carboxyethyl]sulfanyl]acetamido-7-methoxy-3-[2-(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate heptahydrate [*88641-36-5*]

Cefminox Sodium Hydrate contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg of cefminox ($C_{16}H_{21}N_7O_7S_3$: 519.58), calculated on the anhydrous basis.

Description Cefminox Sodium Hydrate appears as white to pale yellowish white crystalline powder.
Cefminox Sodium Hydrate is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cefminox Sodium Hydrate and Cefminox Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefminox Sodium Hydrate and Cefminox Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefminox Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 30) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal, A, at around δ 3.2 ppm, a single signal, B, at around δ 3.5 ppm, a single signal, C, at around δ 4.0 ppm, and a single signal, D, at around δ 5.1 ppm. The ratio of the integrated intensity of each signal, A : B : C : D, is about 2 : 3 : 3 : 1.

(4) A solution of Cefminox Sodium Hydrate (1 in 100) responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +62 ~ +72° (50 mg, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.70 g of Cefminox Sodium Hydrate in 10 mL of water is between 4.5 and 6.0.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefminox Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 2.0 g of Cefminox Sodium Hydrate according to Method 3, and perform the test (not more than 1 ppm).

Water 18.0 ~ 20.0 % (0.1 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cefminox Sodium Hydrate is used in a sterile preparation.

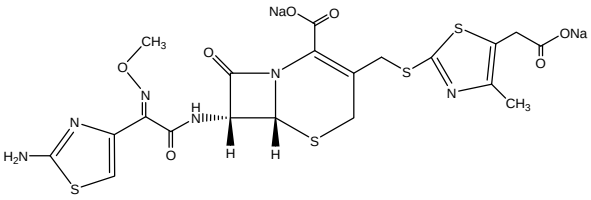
Bacterial Endotoxins Less than 0.05 EU/mg (potency) of cefminox, when Cefminox Sodium Hydrate is used in a sterile preparation.

Assay ***Ultraviolet-visible Spectrophotometry*** Weigh accurately about 70 mg (potency) of Cefminox Sodium Hydrate, dissolve in water to make exactly 100 mL, and use this solution as the test stock solution. Pipet 5 mL of the test stock solution, add 0.1 mol/L acetate buffer solution (pH 4.0) to make exactly 100 mL, and use this solution as test solution I. Separaetly, pipet 5 mL of the test stock solution, add 5.0 mL of hydroxylamine hydrochloride-acetic acid TS, allow to stand for 30 minutes, add 0.1 mol/L acetate buffer solution (pH 4.0) to make exactly 100 mL, and use this solution as test solution II. Separaetly, weigh accurately about 70 mg (potency) of Cefminox Sodium RS, dissolve in water to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add 0.1 mol/L acetate buffer solution (pH 4.0) to make exactly 100 mL, and use this solution as standard solution I. Separately, pipet 5 mL of the standard stock solution, add 5 mL of hydroxylamine hydrochloride-acetic acid TS, allow to stand for 30 minutes, add 0.1 mol/L acetate buffer solution (pH 4.0) to make exactly 100 mL, and use this solution as standard solution II. Determine the absorbances, A_{TI}, A_{TII}, A_{SI}, and A_{SII}, at 273 nm of test solutions I and II and standard solutions I and II as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount [μg (potency)] of cefminox in 1 mg of Cefminox Sodium Hydrate} = \frac{A_{TI} - A_{TII}}{A_{SI} - A_{SII}} \times \frac{\text{Amount [}\mu\text{g (potency)] of Cefminox Sodium RS taken}}{\text{Amount (mg) of Cefminox Sodium Hydrate taken}}$$

Containers and Storage ***Containers***—Hermetic containers.

Cefodizime Sodium



$C_{20}H_{18}N_6Na_2O_7S_4$: 628.63

Disodium (6*R*,7*R*)-7-{2-[(2*E*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino]acetamido}-3-{[(5-carboxylatomethyl)-4-methyl-1,3-thiazol-2-yl]sufanyl}methyl-3,4-didehydrocepham-4-carboxylate [*86329-79-5*]

Cefodizime Sodium contains not less than 890 μg (potency) per mg, calculated on the anhydrous basis and corrected by the ethanol amount.

Description Cefodizime Sodium appears as white to pale yellowish white crystalline powder.
Cefodizime Sodium is very soluble in water, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Cefodizime Sodium and Cefodizime Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefodizime Sodium and Cefodizime Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefodizime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trymethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B, and C at around δ 2.3 pp, at around δ 4.0 ppm, and at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A : B : C, is about 3 : 3 : 1.

(4) Cefodizime Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -56 ~ -62° (0.2 g calculated on the anhydrous basis and corrected by the ethanol amount, water, 20 mL, 100 mm)

pH Dissolve 1.0 g (potency) of Cefodizime Sodium in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the solution is clear and pale yellow to light yellow.

(2) ***Heavy metals***—Weigh 1.0 g of Cefodizime Sodium in a porcelain crucible, cover loosely, and carbonize by gentle heating. After cooling, add 2 mL of sulfuric acid, heat gradually until the white fumes are no longer evolved, and ignite to incinerate between 500 °C and 600 °C. Proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefodizime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 30 mg of Cefodizime Sodium in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefodizime from the test solution is not larger than the peak area of cefodizime from the standard solution, and the total area of the peaks other than cefodizime from the test solution is not larger than 3 times the peak area of cefodizime from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefodizime obtained from 5 μL of this solution is equivalent to 7 to 13 % of that from 5 μL of the standard solution.

Time span of measurement: About 4 times as long as the retention time of cefodizime beginning after the solvent peak.

(5) ***Ethanol***—Weigh accurately about 1 g of Cefodozime Sodium, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this

solution as the test solution. Separately, weigh accurately about 2 g of ethanol (95), and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard. Calculate the amount of ethanol according to the following equation: not more than 2.0 %.

$$\text{Amount (\%) of ethanol} = \frac{\text{Amount [g (potency)] of ethanol}}{W_T} \times \frac{Q_T}{Q_S}$$

W_T: Amount (g) of Cefodizime Sodium taken

Internal standard solution—A solution of 1-propanol (1 in 400)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A glass column about 3.2 mm internal diameter and about 3 m in length, packed with tetrafluoroethylene polymer for gas chromatography (180 to 250 μm in particle diameter) coated with polyethylene glycol 20M for gas chromatography at the rate of 15 %.

Column temperature: A constant temperature of about 100 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 3 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0 %.

Water Not more than 4.0 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cefodizime Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefodizime, when Cefodizime Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefodizime Sodium and Cefodizime Sodium RS, dissolve in exactly 10 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefodizime to that of the internal standard.

$$\text{Amount [μg (potency)] of cefodizime } (C_{20}H_{20}N_6O_7S_4) = \text{Amount [μg (potency) of Cefodizime Sodium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of anhydrous caffeine (3 in 400)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.80 g of potassium dihydrogen phosphate and 0.20 g of anhydrous disodium hydrogen phosphate in water, add 80 mL of acetonitrile, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefodizime is about 5 minutes.

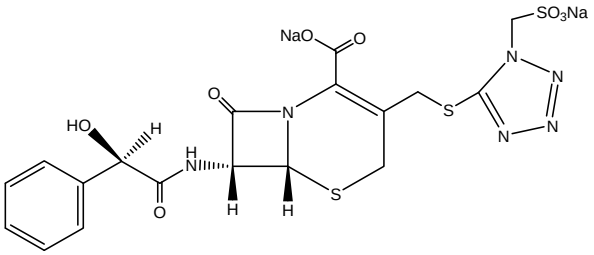
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefodizime and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefodizime to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefonicid Sodium



$C_{18}H_{16}N_6Na_2O_8S_3$: 586.53

Disodium (6*R*,7*R*)-7-[(2*R*)-2-hydroxy-phenylacetamido]-3-[[1-(sufonatomethyl)tetrazol-5-yl]sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*61270-78-8*]

Cefonicid Sodium contains not less than 832 μg (potency) and not more than 970 μg (potency) per mg of cefonicid ($C_{18}H_{18}N_6O_8S_3$: 542.57), calculated on the anhydrous basis.

Description Cefonicid Sodium appears as pale white masses.

Identification (1) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(2) Cefonicid Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -37 ~ -47° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving an amount of Cefonicid Sodium, equivalent to 1.0 g (potency) of cefonicid, in 20 mL of water is between 3.5 and 6.5.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefonicid Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.35 EU/mg (potency) of cefonicid, when Cefonicid Sodium is used in a sterile preparation.

Assay Weigh accurately about 40 mg (potency) each of Cefonicid Sodium and Cefonicid Sodium RS, dissolve separately in the mobile phase to make exactly 200 mL, pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefonicid in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefonicid } (C_{18}H_{18}N_6O_8S_3) = \text{Amount [μg (potency)] of Cefonicid Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 300 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of water, methanol, and 0.2 mol/L ammonium dihydrogen phosphate solution (33 : 5 : 2)

Flow rate: About 2.0 mL/minute

System suitability

Resolution test solution: Dissolve a suitable amount of Cefonicid Sodium RS in the mobile phase to make a solution so that each mL contains about 0.2 mg (potency). Warm this solution in a water bath for 30 minutes, and cool. This solution contains cefonicid and diacetyl cefonicid.

System performance: When the procedure is run with 10 μL each of the standard solution and resolution test solution under the above operating conditions, the resolution between the peaks of cefonicid and diacetyl cefonicid is not less than 1.1, and the number of theoretical plates and the symmetry factor are not less than 1500 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefonicid is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefonicid Sodium for Injection

Cefonicid Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefonicid Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefonicid ($C_{18}H_{18}N_6O_8S_3$: 542.57).

Method of Preparation Prepare as directed under Injections, with Cefonicid Sodium.

Description Cefonicid Sodium for Injection appears as white to pale white powder.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Specific Optical Rotation $[\alpha]_D^{20}$: -37 ~ -47° [0.1 g (potency) calculated on the anhydrous basis, methanol, 10 mL, 100 mm]

pH The pH of a solution obtained by dissolving an amount of Cefonicid Sodium for Injection, equivalent to 1 g (potency) of cefonicid, in 20 mL of water is between 3.5 and 6.5.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.35 EU/mg (potency) of cefonicid.

Foreign Insoluble Matter Test It meets the requirement.

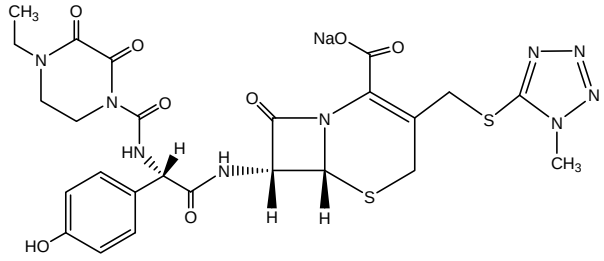
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefonicid Sodium. Weigh accurately a suitable amount of Cefonicid Sodium for Injection, dissolve in the mobile phase so that each mL contains 20 μg (potency), and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$: 667.65

Sodium (6*R*,7*R*)-7-[[(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-(4-hydroxyphenyl)acet]amido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*62893-20-3*]

Cefoperazone Sodium contains not less than 871 μg (potency) per mg of cefoperazone ($C_{25}H_{27}N_9O_8S$: 645.67), calculated on the anhydrous basis.

Description Cefoperazone Sodium is a white to yellowish white crystalline powder.
Cefoperazone Sodium is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Cefoperazone Sodium and Cefoperazone RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefoperazone Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate as an internal reference compound: it exhibits a triplet signal, A, at around δ 1.2 ppm, and double signals, B and C, at around δ 6.8 ppm and at around δ 7.3 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C, is 3 : 2 : 2.

(3) Cefoperazone Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement, except for freeze-dried powder.

Specific Optical Rotation $[\alpha]_D^{20}$: -15 ~ -25° (1 g, water, 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Cefoperazone Sodium in 4 mL of water is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefoperazone Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Cefoperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately 0.1 g of Cefoperazone Sodium, add water to make 100 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas of each solution by the automatic integration method, and calculate the percentage of each related substance from the test solution to 50 times the peak area of cefoperazone from the standard solution: the related substance with the retention time of about 8 minutes is not more than 5.0 %, the related substance with that of about 17 minutes is not more than 1.5 %, and the total of all related substances is not more than 7.0 %. Use the peak areas of the related substances with the retention time of about 8 minutes and 17 minutes after multiplying by their relative response factors, 0.90 and 0.75, respectively.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operat-

ing conditions in the Assay.

System suitability

Detection sensitivity: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefoperazone obtained from 25 μL of this solution is equivalent to 3.5 to 6.5 % of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefoperazone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefoperazone is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of cefoperazone beginning after the solvent peak.

Water Crystalline powder: not more than 5.0 % (0.2 g, volumetric titration, direct titration). Freeze-dried powder: not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cefoperazone Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of cefoperazone, when Cefoperazone Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) of Cefoperazone Sodium, dissolve in water to make exactly 100 mL, pipet 5 mL of this solution, add 5 mL of the internal standard solution, and use this solution as the test solution. Separately, weigh accurately about 0.1 g (potency) of Cefoperazone RS, dissolve in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefoperazone to that of the internal standard.

$$\text{Amount [μg (potency)] of cefoperazone } (C_{25}H_{27}N_9O_8S) = \text{Amount [μg (potency)] of Cefoperazone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43 : 7) (3 in 8000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 35 °C

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine, add water to make 1000 mL. To 20 mL of this solution add 835 mL of water, 140 mL of acetonitrile, and 5 mL of dilute acetic acid.

Flow rate: Adjust the flow rate so that the retention time of cefoperazone is about 10 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operation conditions, the internal standard and cefoperazone are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the operating conditions, the relative standard deviation of the ratios of the peak area of cefoperazone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In a cold place.

Cefoperazone Sodium for Injection

Cefoperazone Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefoperazone Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefoperazone ($C_{25}H_{27}N_9O_8S_2$: 645.67).

Method of Preparation Prepare as directed under Injections, with Cefoperazone Sodium.

Description Cefoperazone Sodium for Injection appears as white to yellowish white powder.

Identification (1) Determine the infrared spectra of Cefoperazone Sodium for Injection and Cefoperazone RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve a suitable amount each of Cefoperazone Sodium for Injection and Cefoperazome in a mixture of acetone and water (9 : 1) to make solutions so that each mL contains 1.0 mg, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-

layer chromatography. Develop the plate with a mixture of 2-butanone, acetic acid (100), and water (18 : 3 : 3), and examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution and standard solution show the same R_f value.

(3) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving an amount of Cefoperazone Sodium for Injection, equivalent to 0.25 g (potency) of cefoperazone, in 1 mL of water is between 4.5 and 6.5.

Water Crystalline powder: Not more than 5.0 % (0.2 g, volumetric titration, direct titration). Freeze-dried powder: Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of cefoperazone.

Foreign Insoluble Matter Test It meets the requirement.

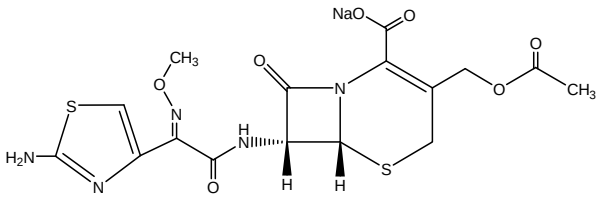
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefoperazone Sodium. Weigh accurately an amount of Cefoperazone Sodium for Injection, equivalent to about 0.1 g (potency) according to the labeled potency, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefotaxime Sodium



$C_{16}H_{16}N_5NaO_7S_2$: 477.45

Sodium (6*R*,7*R*)-7-{2-[(2*E*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino]acetamido}-3-acetyloxymethyl-3,4-didehydrocepham-4-carboxylate [*64485-93-4*]

Cefotaxime Sodium contains not less than 916 μg (potency) per mg of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47), calculated on the dried basis.

Description Cefotaxime Sodium is a white to light yellowish white crystalline powder.
Cefotaxime Sodium is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Dissolve separately 2 mg each of Cefotaxime Sodium and Cefotaxime Sodium RS in 0.01 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefotaxime Sodium and Cefotaxime Sodium RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the ^1H spectrum of a solution of Cefotaxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, and C, at around δ 2.1 ppm, at around δ 4.0 ppm, and around δ 7.0 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C, is about 3 : 3 : 1.

(4) Cefotaxime Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +58 ~ +64° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1 g of Cefotaxime Sodium in 10 mL of water is between 4.5 and 6.5.

Absorbance $E_{1cm}^{1\%}$ (235 nm): 360 ~ 390 (20 mg calculated on the dried basis, water, 1000 mL).

Purity (1) ***Clarity and color of solution***—A solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is clear and light yellow.

(2) ***Sulfate***—Dissolve 2.0 g of Cefotaxime Sodium in 40 mL of water, add 2 mL of dilute hydrochloric acid and water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add water to make 50 mL. Perform the test with this solution as the test solution.

Prepare the control solution as follows: To 1.0 mL of 0.005 mol/L sulfuric acid add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Cefotaxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Proceed with 1.0 g of Cefotaxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(5) ***Related substances***—Perform the test according to the Assay of Cefotaxime Sodium. Weigh accurately about 25 mg of Cefotaxime Sodium, dissolve in the mobile phase to make exactly 25 mL, and use this solution as the test solution. Time span of measurement is about 3.5 times as long as the retention time of cefotaxime beginning after the solvent peak (Each peak area other than cefotaxime is not more than 1.0 % and the total of these peak areas is not more than 3.0 %).

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of cefotaxime obtained from 10 μL of this solution is equivalent to 0.15 to 0.25 % of that from the standard solution.

(6) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cefotaxime Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, if necessary, centrifuge, use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2 mL of hydrochloric acid and water to make 50 mL. Pipet 1.0 mL, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, if necessary, centrifuge, use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard for the test solution and the standard solution, respectively. (not more than 20 ppm)

$$\text{Amount (ppm) of dimethylaniline} = \text{Amount (mg) of dimethylaniline taken} \times \frac{Q_T}{Q_S} \times \frac{\text{Amount (\%) of dimethylan iline}}{\text{Amount (mg) of Cefotaxime Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane and make 50 mL. Pipet 5.0 mL of this solution, add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector.

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 ºC

Injector and detector temperature: 150 ºC

Carrier gas: Nitrogen gas

Flow rate: 30 mL/minute.

Loss on Drying Not more than 3.0 % (1 g, 105 °C, 3 hours).

Sterility Test It meets the requirement, when Cefotaxime Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.05 EU/mg (potency) of cefotaxime, when Cefotaxime Sodium is used in a sterile preparation.

Assay Weigh accurately about 40 mg (potency) each of Cefotaxime Sodium and Cefotaxime Sodium RS, dissolve each in the mobile phase A to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of cefotaxime of these solutions.

$$\text{Amount [μg (potency)] of cefotaxime } (C_{16}H_{17}N_5O_7S_2) = \text{Amount [μg (potency)] of Cefotaxime Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 30 °C

Mobile phase: Control the concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 860 mL of this solution add 140 mL of methanol.

Mobile phase B: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.26. To 600 mL of this solution add 400 mL of methanol.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-7	100	0
7-9	100→80	0→20
9-16	80	20
16-45	80→0	20→100
45-50	0	100

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 14 minutes (about 1.3 mL/minute).

System suitability

System performance: To 1 mL of the standard solution add 7.0 mL of water and 2.0 mL of methanol, mix, then add 25 mg of sodium carbonate decahydrate, and shake. After allowing to stand for 10 minutes, add 3 drops of acetic acid (100) and 1 mL of the standard solution, and shake. When the procedure is run with 10 μL of this solution under the above operating conditions, desacetyl cefotaxime with the relative retention time being about 0.3 to cefotaxime and cefotaxime are eluted in this order with the resolution between these peaks being not less than 20, and the symmetry factor of the peak of cefotaxime is not more than 2.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotaxime is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefotaxime Sodium for Injection

Cefotaxime Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefotaxime Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47)

Method of Preparation Prepare as directed under Injections, with Cefotaxime Sodium.

Description Cefotaxime Sodium for Injection appears as white to light yellowish white powder.

Identification (1) Perform the test as directed in the Identification (2) under Cefotaxime Sodium.

(2) Perform the test as directed in the Identification (4) under Cefotaxime Sodium.

(3) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving an amount of Cefotaxime Sodium for Injection, equivalent to 1.0 g (potency) of cefotaxime, in 10 mL of water is between 4.5 and 6.5.

Purity ***Related substances***—Perform the test as directed in the Purity (5) under Cefotaxime Sodium. The time span of measurement is 8 times the retention time of the peak of the principal component (each related substance is not more than 1.0 %, and the total amount of related substances is not more than 4.0 %).

Loss on Drying Not more than 3.0 % (1 g, 105 °C, 3 hours)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.05 EU/mg (potency) of cefotaxim.

Foreign Insoluble Matter Test It meets the requirement.

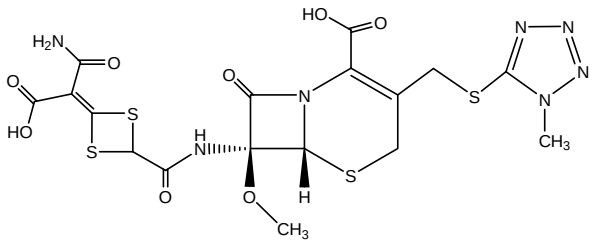
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefotaxime Sodium. Weigh accurately an amount of Cefotaxime Sodium for Injection, equivalent to about 40 mg (potency) according to the labeled potency, dissolve in the mobile phase A to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefotetan



$C_{17}H_{17}N_7O_8S_4$: 575.62

(6*R*,7*S*)-7-[[4-(2-Amino-1-carboxy-2-oxoethylidene)-1,3-dithietane-2-carbonyl]amino]-7-methoxy-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylic acid [*69712-56-7*]

Cefotetan contains not less than 960 μg (potency) and not more than 1010 μg (potency) per mg of cefotetan

($C_{17}H_{17}N_7O_8S_4$: 575.62), calculated on the anhydrous basis.

Description Cefotetan appears as white to pale yellowish white powder.
Cefotetan is sparingly soluble in methanol, and slightly soluble in water or in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cefotetan and Cefotetan RS in 1 % phosphate buffer solution (pH 6.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefotetan and Cefotetan RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Cefotetan in 0.5 mL of a solution of sodium hydrogen carbonate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25). Determine the ^{1}H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals at around δ 3.6 ppm, at around δ 4.0 ppm, at around δ 5.1 ppm, and at around δ 5.2 ppm, and the ratio of the integrated intensity of each signal is 3 : 3 : 1 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +112 ~ +124° [0.5 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 200), 50 mL, 100 mm]

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefotetan in 10 mL of a solution of sodium hydrogen carbonate (1 in 30): the solution is clear, and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefotetan according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 0.1 g of Cefotetan, dissolve in methanol, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the test solution. Separately, weigh about 3 mg of 1-Methyl-1*H*-tetrazole-5-thiole RS for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and about 2 mg of Cefotetan RS, calculated on the anhydrous basis, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_{Ta}, Q_{Tb}, Q_{Tc}, Q_{Td}, Q_{Te} and Q_{Tf}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone having the relative retention time of about 0.5 with respect to cefotetan, *Δ*2-cefotetan having the relative retention time of about 1.2 with respect to cefotetan, isothiazole substance having the relative retention time of about 1.3 with respect to cefotetan, each of other related substances and the total of other related substances, to the peak area of the internal standard, respectively, obtained from the test solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol and cefotetan, to the peak area of the internal standard, respectively, obtained from the standard solution. Calculate the amount of each related substance from the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol is not more than 0.3 %, cefotetan lactone is not more than 0.3 %, *Δ*2-cefotetan is not more than 0.5 %, isothiazole substance is not more than 0.5 %, each of other related substances is not more than 0.2 % and the total of other related substances is not more than 0.4 %.

$$\text{1-Methyl-1}H\text{-tetrazole-5-thiol}\,(\%) = \frac{W_{Sa}}{W_T} \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{100}$$

$$\text{Amount (\%) of cefotetan lactone} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Amount (\%) of } \Delta 2\text{-cefotetan} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tc}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Amount (\%) of isothiazole substance} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Td}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Amount of each of other related substances (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Te}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Total of other related substances}\,(\%) = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tf}}{Q_{Sb}} \times \frac{1}{100}$$

W_{Sa}: Amount (mg) of 1-metyl-1*H*-tetrazole-5-thiol
W_{Sb}: Amount (mg) of Cefotetan RS, calculated on the anhydrous basis
W_T: Amount (g) of Cefotetan

Internal standard solution—A solution of anhydrous caffeine in methanol (3 in 10000)

Operating conditions
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.
System suitability
System performance: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Pipet 15 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of cefotetan obtained from 5 μL of this solution is equivalent to 12 to 18 % of that from the standard solution.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating condition, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 2.0 %.

Time span of measurement: About 3.5 times as long as the retention time of cefotetan.

Isomer Ratio Dissolve 10 mg of Cefotetan in 20 mL of methanol, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the area of the adjacent two peaks that appear at around the retention time of 40 minutes, one having a shorter retention time (*l*-substance) and another having a longer retention time (*d*-substance). Calculate the amount of *l*-substance by the area percentage method: not less than 35 % and not more than 45 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution (pH 7.0), water, and a solution of tetrabutylammonium hydrogensulfate in acetonitrile (1 in 150) (9 : 9 : 2)

Flow rate: Adjust the flow rate so that the retention time of *l*-substance is about 40 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the test solution under the above operating conditions, *l*-substance and *d*-substance are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: To exactly 1 mL of the test solution add methanol to make exactly 10 mL. When the test is repeated 6 times with 5 μL each of this solution, the relative standard deviation of the peak area of *l*-substance is not more than 5.0 %.

Water Not more than 2.5 % (1 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (1 g)

Sterility Test It meets the requirement, when Cefotetan is used in a sterile preparation.

Bacterial Endotoxins Less than 0.17 EU/mg (potency) of cefotetan, when Cefotetan is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefotetan and Cefotetan RS, and dissolve each in 1 % phosphate buffer solution (pH 6.5) to make exactly 50 mL. Pipet 15 mL each of these solutions, add exactly 10 mL of the internal standard solution and 1 % phosphate buffer solution (pH 6.5) to make exactly 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefotetan to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefotetan } (C_{17}H_{17}N_7O_8S_4) = \text{Amount [μg (potency)] of Cefotetan RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of anhydrous caffeine (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 11.53 g of phosphoric acid in 1000 mL of water. To 850 mL of this solution add 50 mL of acetonitrile, 50 mL of acetic acid (100) and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefotetan is about 17 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, caffeine and cefotetan are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is performed with 5 μL each of the standard solution, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5 °C.

Cefotetan Sodium for Injection

Cefotetam Sodium for Injection is a preparation for injection, which is dissolved before use.

Cefotetam Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefotetan ($C_{17}H_{17}N_7O_8S_4$: 575.62).

Method of Preparation Prepare as directed under Injections, with Cefotetan by add sodium hydrogen carbonate to make cefotetan disodium.

Description Cefotetan Sodium for Injection appears as white to pale yellowish white powder.

Identification (1) Weigh an amount of Cefotetan Sodium for Injection, equivalent to about 10 mg (potency) of cefotetan, dissolve in 2 mL of hydroxylamine hydrochloride TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Weigh an amount of Cefotetan Sodium for Injection, equivalent to 10 mg (potency) of cefotetan, and dissolve in 1 % phosphate buffer solution (pH 6.5) to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 283 nm and 287 nm.

(3) Determine the infrared spectrum of Cefotetan Sodium for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 1760 cm^{-1}, 1630 cm^{-1}, 1520 cm^{-1}, and 1080 cm^{-1}.

pH The pH of a solution obtained by dissolving an amount of Cefotetan Sodium for Injection, equivalent to 0.1 g (potency) of cefotetan, in 1 mL of water is between 4.5 and 6.5.

Water Not more than 1.5 % (1.0 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.17 EU/mg (potency) of cefotetan.

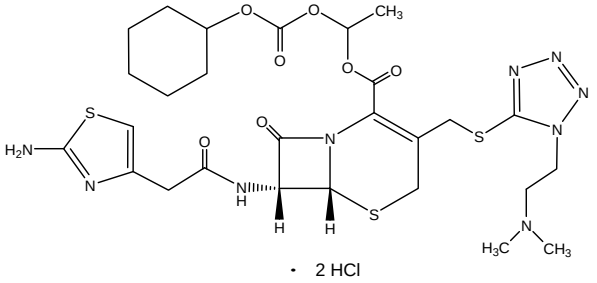
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefotetan. Weigh a suitable amount of Cefotetan Sodium for Injection, and dilute with 1 % phosphate buffer solution (pH 6.5) to make a solution so that each mL contains 1 mg (potency). Pipet 15 mL of this solution, add exactly 10 mL of the internal standard solution and 1 % phosphate buffer solution (pH 6.5) to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefotiam Hexetil Hydrochloride



· 2 HCl

$C_{27}H_{37}N_9O_7S_3 \cdot 2HCl$: 768.76

1-Cyclohexyloxycarbonyloxyethyl (6*R*,7*R*)-7-[[2-(2-amino-1,3-thiazol-4-yl)acet]amido]-3-[[1-[2-(dimethylamino)ethyl]tetrazol-5-yl]sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate dihydrochloride [*95789-30-3*]

Cefotiam Hexetil Hydrochloride contains not less than 615 μg (potency) and not more than 690 μg (potency) per mg of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63), calculated on the anhydrous basis.

Description Cefotiam Hexetil Hydrochloride appears as white to pale yellowish white powder.
Cefotiam Hexetil Hydrochloride is very soluble in water, in methanol, or in ethanol (95), freely soluble in dimethylsulfoxide, and slightly soluble in acetonitrile.
Cefotiam Hexetil Hydrochloride dissolves in 0.1 mol/L hydrochloric acid TS.
Cefotiam Hexetil Hydrochloride is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Cefotiam Hexetil Hydrochloride and Cefotiam Hexetil Hydrochloride RS in 1 mol/L hydrochloric acid TS (3 in 125000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelgnths.

(2) Determine the ^1H spectrum of a solution of Cefotiam Hexetil Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 2.8 ppm and at around δ 6.6 ppm, and a multiple signal, C, at around δ 6.9 ppm. The ratio of the integrated intensity of each signal, A : B : C, is about 6 : 1 : 1.

(3) To a solution of Cefotiam Hexetil Hydrochloride (1 in 200) add 2 mL of dilute nitric acid and 1 mL of silver nitrate TS, and mix: a white precipitate is formed.

Specific Optical Rotation $[\alpha]_D^{20}$: +52 ~ +60° (0.1 g

calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Proceed with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 1 ppm).

(3) ***Related substance I***—Weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride RS, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of related substances by the following equation: the amount of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, is not more than 2.0 %, and each amount of the other related substances is not more than 0.5 %. For this calculation, use the peak area obtained by the automatic integration method of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, after multiplying by its relative response factor, 0.78.

$$\text{Amount (\%) of each related substance} = \frac{\text{Amount [g (potency)] of Cefotiam Hexetil Hydrochlor ide RS}}{W_T} \times \frac{A_T}{A_S} \times 5$$

W_T: Amount (g) of Cefotiam Hexetil Hydrochloride taken

A_S: Total of two peak areas of cefotiam hexetil from the standard solution

A_T: Peak area of each related substance from the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust so that the mixing rate of the mobile phase A and the mobile phase B is changed lineally from 1 : 0 to 0 : 1 for 30 minutes.

Mobile phase A: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate (TS (1 in 2), acetonitrile, and acetic acid (100) (72 : 28 : 1).

Mobile phase B: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate (TS (1 in 2), acetonitrile, and acetic acid (100) (60 : 40 : 1).

Flow rate: 0.7 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make exactly 50 mL. Confirm that each area of the two peaks of cefotiam hexetil obtained from 10 μL of this solution is equivalent to 1.6 to 2.4 % of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefotiam hexetil is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the total of the two peak areas of cefotiam hexetil is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of one of the cefotiam hexetil peaks, which appears first, beginning after the solvent peak.

(4) ***Related substance II***—Weigh accurately about 20 mg of Cefotiam Hexetil Hydrochloride, dissolve in exactly 2 mL of methanol, add a mixture of a solution of diammonium hydrogen phosphate (79 in 20,000) and acetic acid (100) (200 : 3) to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg of Cefotiam Hydrochloride RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amounts of the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam are not more than 1.0 %, respectively, and each amount of the related substances other than the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam is not more than 0.5 %. For this calculation, use the peak area of the related substance having the relative retention time of about

0.9 to cefotiam after multiplying by its relative response factor, 0.76.

$$\text{Amount (\%) of each related substance} = \frac{\text{Amount [g (potency)] of Cefotiam Hexetil Hydrochlor ide RS}}{W_T} \times \frac{A_T}{A_S} \times 4$$

W_T: Amount (g) of Cefotiam Hexetil Hydrochloride taken

A_S: Peak area of cefotiam from the standard solution

A_T: Peak area of each related substance from the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (79 in 20000), methanol, and acetic acid (100) (200 : 10 : 3)

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 15 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of cefotiam obtained from 10 μL of this solution is equivalent to 1.6 to 2.4 % of the peak area of cefotiam from the standard solution.

System performance: To 1 mL of a solution of acetoaminophen in the mobile phase (1 in 50000) add 3 mL of the standard solution, and mix well. When the procedure is run with 10 μL of this solution under the above operating conditions, acetoaminophen and cefotiam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotiam is not more than 2.0 %.

(5) ***Total related substances***—The total of the amount of related substances obtained in the Related substance I and the Related substance II is not more than 6.5 %.

Water Not more than 3.5 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Isomer Ratio Perform the test with 20 μL of the test solution used in the Assay as directed in the Assay, and determine the areas of the two peaks, A_a for the faster peak and A_b for the later peak, appeared at the retention time of about 10 minutes: $A_a/(A_a+A_b)$ is not less than 0.45 and not more than 0.55.

Assay Weigh accurately about 30 mg (potency) each of Cefotiam Hexetil Hydrochloride, and dissolve each in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make exactly 50 mL. To 5 mL of each solution add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefotiam hexetil to that of the internal standard in each solution. For this calculation, the total of the areas of the two peaks appeared at the retention time of about 10 minutes is used as the peak area of cefotiam hexetil.

$$\text{Amount [μg (potency)] of cefotiam } (C_{18}H_{23}N_9O_4S_3) = \text{Amount [μg (potency)] of Cefotiam Hexetyl Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzoic acid in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4 : 1) (7 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile, and acetic acid (100) (72 : 28 : 1)

Flow rate: Adjust the flow rate so that the retention time of the faster peak of cefotiam hexetil is about 9 minutes.

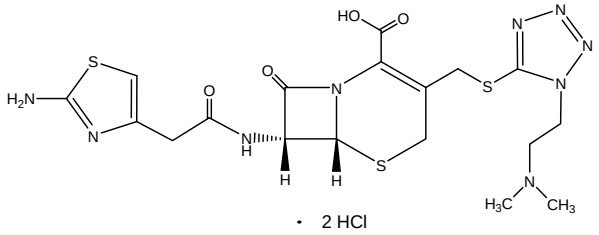
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and cefotiam hexetil are eluted in this order with the resolution between the two peaks of cefotiam hexetil being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotiam hexetil to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefotiam Hydrochloride



· 2 HCl

$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$: 598.55

(6*R*,7*R*)-7-[[2-(2-Amino-1,3-thiazol-4-yl)acet]amido]-3-[[1-[2-(dimethylamino)ethyl]tetrazol-5-yl]sulfanylmethyl]-3,4-didehydrocepham-4-carboxylic acid dihydrochloride [*66309-69-1*]

Cefotiam Hydrochloride contains not less than 810 μg (potency) and not more than 890 μg (potency) per mg of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63), calculated on the anhydrous basis.

Description Cefotiam Hydrochloride appears as white to pale yellowish white crystals or crystalline powder.
Cefotiam Hydrochloride is freely soluble in water, in ethanol, or in formamide, slightly soluble in ethanol, and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Cefotiam Hydrochloride and Cefotiam Hydrochloride RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefotiam Hydrochloride and Cefotiam Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefotiam Hydrochloride in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.1 ppm and at around δ 6.7 ppm, respectively. The ratio of the integrated intensity of each signal, A : B, is about 6 : 1.

(4) Dissolve 0.1 g of Cefotiam Hydrochloride in 5 mL of dilute nitric acid, and immediately add 1 mL of silver nitrate TS: a white precipitate is formed.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +60 ~ +72° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm)

pH Dissolve 1.0 g (potency) of Cefotiam Hydrochloride in 10 mL of water: the pH of the solution is between 1.2 and 1.7.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the solution is clear, and colorless to yellow.

(2) ***Heavy metals***—To about 1.0 g of Cefotiam Hydrochloride add 1 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, then heat gradually to incinerate. If a carbonized residue still remains, moisten the residue with a small amount of sulfuric acid, and ignite again to incinerate. After cooling, add 2 mL of hydrochloric acid to the residue, heat on a water bath to dissolve, then heat to dryness. Add 10 mL of water, and heat on a water bath to dissolve. After cooling, add ammonia TS dropwise to adjust the pH to 3 to 4, if necessary, filter, wash the residue with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution in the same manner as for preparation of the test solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefotiam Hydrochloride according to Method 4, and perform the test. After cooling, add 10 mL of dilute hydrochloric acid to the residue (not more than 2 ppm).

Water Not more than 7.0 % (0.25 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination).

Sterility Test It meets the requirement, when Cefotiam Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.125 EU/mg (potency) of cefotiam, when Cefotiam Hydrochloride is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Cefotiam Hydrochloride and Cefotiam Hydrochloride RS, dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefotiam.

Amount [μg (potency)] of cefotiam ($C_{18}H_{23}N_9O_4S_3$)

$$= \text{Amount [μg (potency)] of Cefotiam RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 800 mL of 0.025 mol/L disodium hydrogenphosphate TS add 0.01 mol/L potassium diihydrogenphosphate TS to adjust the pH to 7.7. To 440 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 14 minutes.

System suitability

System performance: Dissolve 0.04 g of orcine in 10 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, orcine and cefotiam are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak areas of cefotiam is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Cefotiam Hydrochloride for Injection

Cefotiam Hydrochloride for Injection is a preparation for injection which is dissolved before use.
Cefotiam Hydrochloride for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Method of Preparation Prepare as directed under Injections, with Cefotiam Hydrochloride.

Description Cefotiam Hydrochloride for Injection appears as white to pale yellow powder.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hydrochloride for Injection (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 257 nm and 261 nm.

(2) Determine the ^1H spectrum of a solution of Cefotiam Hydrochloride for Injection in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals, A and B, at between δ 2.7 and δ 3.0 ppm and at around δ 6.5 ppm, respectively. The ratio of the integrated intensity of each signal, A : B, is about 6 : 1.

pH The pH of a solution obtained by dissolving an amount of Cefotiam Hydrochloride for Injection, equivalent to 0.5 g (potency), in 5 mL of water is between 5.7 and 7.2.

Purity ***Clarity of solution***—Dissolve an amount of Cefotiam Hydrochloride for Injection, equivalent to 1.0 g (potency) of cefotiam hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 450 nm 10 minutes after dissolving as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.20.

Loss on Drying Not more than 6.0 % (0.5 g, in vacuum, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.125 EU/mg (potency) of cefotiam.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

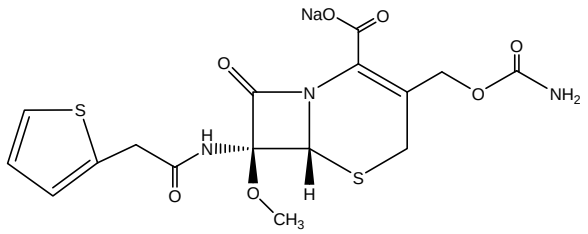
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 10 containers of Cefotiam Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of cefotiam hydrochloride according to the labeled amount, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Cefotiam Hydrochloride RS, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefotiam Hydrochloride.

$$\text{Amount [μg (potency)] of cefotiam } (C_{18}H_{23}N_9O_4S_3) = \text{Amount [μg (potency)] of Cefotiam RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefoxitin Sodium



$C_{16}H_{17}N_3NaO_7S_2$: 449.44

Sodium (6*R*,7*R*)-7-[2-(thiophen-2-yl)acetamido]-3-carbamoyloxymethyl-3,4-didehydrocepham-4-carboxylate [*33564-30-6*]

Cefoxitin Sodium contains not less than 927 μg (potency) and not more than 970 μg (potency) per mg of cefoxitin ($C_{16}H_{17}N_3O_7S_2$: 427.46), calculated on the anhydrous basis and corrected by the amount of acetone and methanol.

Description Cefoxitin Sodium is a white to pale yellowish white particle or powder.
Cefoxitin Sodium is very soluble in water, soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(2) Weigh 25 mg of Cefoxitin Sodium, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 and make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry, it exhibits maxima between 234 nm and 238 nm, and between 260 nm and 264 nm.

(3) Weigh 20 mg each of Cefoxitin Sodium and Cefoxitin RS, dissolve in methanol to make 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the developing solvent which is a mixture of chloroform, acetone, and formic acid (10:9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS on the plate and heat the plate at about 80 °C for 5 minutes. The red-purple spots obtained from the test solution and the standard solutions are the same in the R_f value.

(4) Cefoxitin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +206 ~ +214° (0.25 g calculated on the anhydrous basis and corrected by the amount of acetone and methanol, methanol, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1 g of Cefoxitin Sodium in 10 mL of water is between 4.2 and 7.0.

Absorbance $E_{1cm}^{1\%}$ (262 nm): 190 ~ 210(2.0 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 100 mL).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefoxitin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Acetone and methanol***—Dissolve 5.0 g of Cefoxitine Sodium in water to make exactly 50 mL. Put 3.0 mL of this solution in a centrifuge tube, cool in ice water for 2 minutes, add 3.0 mL of 0.24 mol/L hydrochloric acid with vigorous shaking, centrifuge, and use the clear supernatant liquid as the test solution. Separately, to 5.0 mL of acetone add water to make exactly 1000 mL, and use this solution as the solution (1). To 5.0 mL of methanol add methanol to make 1000 mL, and use this solution as the solution (2). To 50.0 mL of the solution (1) and 5.0 mL of the solution (2), add water to make exactly 500 mL, and use this solution as the standard solution. The concentrations of acetone and methanol in this solution are 0.050 % and 0.005 % (v/v), respectively. Perform the test with 2 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following operating conditions, and determine the peak areas of acetone and methanol in the test solution and standard solution, A_U and A_S. Calculate the amount of acetone and methanol by the following equation: not more than 0.7 % of acetone and not more than 0.1 % of methanol.

$$\text{Amount (\%) of acetone and methanol} = \frac{D \cdot P}{C \times \dfrac{A_U}{A_S}}$$

D: Specific gravity (g/mL) of acetone and methanol at a temperature of 20 °C

P: Concentration (v/v %) of acetone and methanol in the standard solution

C: Concentration (g/mL) of cefoxitin sodium in the test solution

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A glass column about 6.3 mm in internal diameter and about 1.8 m in length, packed with styrene-divinylbenzene copolymer for gas chromatography (average pore size: 0.3 to 0.4 μm, specific surface area: not more than 50 m^2/g).

Colulmn temperature: A constant temperature of about 110 °C

Injection port temperature: 100 °C
Detector temperature: 200 °C
Carrier gas: Nitrogen
Flow rate: 50 mL/minute
System suitability

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the numbers of theoretical plates of the peaks of acetone and ethanol are not less than 160 and not less than 200, and the symmetry factors are not more than 1.3 and not more than 2.3, respectively.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone and ethanol is not more than 5.0 %.

(3) ***Cefoxitin lactone***—Weigh accurately about 0.1 g of Cefoxitin Sodium, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the developing solvent which is a mixture of chloroform, acetone, and formic acid (10:9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnam aldehyde TS on the plate and heat the plate at about 80 °C for 5 minutes. The spots other than the principal spot obtained from the test solution are not more intense than those obtained from the standard solution (Not more than 1.0 %).

Water Not more than 1.0 % (0.5 g, volumetric titration, direct titration). Use a mixture of ethylene glycol and pyridine (3 : 1) instead of methanol.

Sterility Test It meets the requirement, when Cefoxitin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg of cefoxitin, when Cefoxitin Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) of Cefoxitin Sodium, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg (potency) of Cefoxitin RS, dissolve in water to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of cefoxitin of these solutions.

Amount [μg (potency)] of cefoxitin ($C_{16}H_{17}N_3O_7S_2$)

$$= \text{Amount [μg (potency)] of Cefoxitin RS} \times \frac{A_T}{A_S}$$

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm)
Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)
Mobile phase: A mixture of water, methanol and acetic acid (100) (50 : 50 : 1)
Flow rate: Adjust the flow rate so that the retention time of cefoxitin is about 6 minutes.

Containers and Storage ***Containers***—Tight containers.

Cefoxitin Sodium for Injection

Cefoxitin Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefoxitin Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$: 427.46).

Method of Preparation Prepare as directed under Injections, with Cefoxitin Sodium.

Description Cefoxitin Sodium for Injection appears as white to pale yellowish white powder.

Identification Perform the test as directed in the Identification (1), (2), and (3) under Cefoxitin Sodium.

pH The pH of a solution obtained by dissolving an amount of Cefoxitin Sodium for Injection, equivalent to 1.0 g (potency) of cefoxitin sodium, in 10 mL of water is between 4.2 and 7.0.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefoxitin.

Foreign Insoluble Matter Test It meets the requirement.

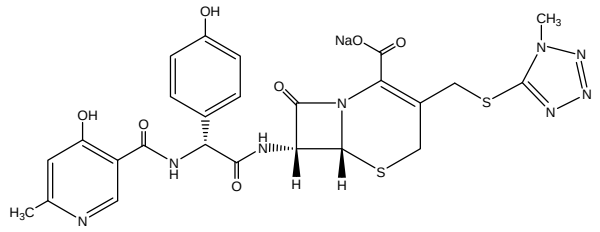
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefoxitin Sodium. Weigh accurately an amount of Cefoxitin Sodium for Injection, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefpiramide Sodium



$C_{25}H_{23}N_8NaO_7S_2$: 634.62

Sodium (6*R*,7*R*)-7-[[(2*R*)-2-(4-hydroxyphenyl)-2-[(4-hydroxy-6-methylpyridin-3-yl)carbonylamino]acetamido]-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-3,4-didehydrocepham-4-carboxylate [*74849-93-7*]

Cefpiramide Sodium contains not less than 900 μg (potency) and not more than 990 μg (potency) per mg of cefpiramide ($C_{25}H_{24}N_8O_7S_2$: 612.64), calculated on the anhydrous basis.

Description Cefpiramide Sodium appears as white to yellowish white powder.
Cefpiramide Sodium is very soluble in dimethylsulfoxice, freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution (pH 7.0) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 270 nm and 275 nm.

(2) Determine the ^{1}H spectrum of a solution of Cefpiramide Sodium in deuterated dimethylsulfoxide (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals at around 2.3 ppm, at around 3.9 ppm, and at around 8.2 ppm. The ratio of the integrated intensity of each signal is about 3 : 3 : 1.

(3) Cefpiramide Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -33 ~ -40° (0.2 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution (pH 7.0), 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1 g (potency) of Cefpiramide Sodium in 10 mL of water is between 5.5 and 8.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution (pH 7.0): the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefpiramide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 25 mg of Cefpiramide Sodium, dissolve in 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg of 1-Methyl-1*H*-tetrazol-5-thiol RS, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and an amount of Cefpiramide RS, equivalent to about 75 mg (potency), and dissolve in 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 100 mL. Pipet 2 mL of this solution, add 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution. Calculate the amount of each related substance by the following equations: the amount of 1-methyl-1*H*-tetrazol-5-thiol, each of other related substances and the total of other related substances are not more than 1.0 %, not more than 1.5 % and not more than 4.0 %, respectively.

$$\text{Amount (\%) of 1-methyl-1}H\text{-tetrazol-5-thiol } (C_2H_4N_4S) = \frac{W_{Sa}}{W_T} \times \frac{A_{Ta}}{A_{Sa}}$$

$$\text{Amount (\%) of each of other related substances} = \frac{W_{Sb}}{W_T} \times \frac{A_{Tc}}{A_{Sb}}$$

W_{Sa}: Amount (mg) of 1-methyl-1*H*-tetrazol-5-thiol
W_{Sb}: Amount [mg (potency)] of Cefpiramide Sodium RS
W_T: Amount (mg) of cefpiramide sodium
A_{Sa}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the standard solution
A_{Sb}: Peak area of cefpiramide from the standard solution
A_{Ta}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the test solution
A_{Tc}: Area of each peak other than 1-methyl-1*H*-tetrazol-5-thiol and cefpiramide from the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.03 mol/L phosphate buffer solution (pH 7.5) and methanol (3 : 1)

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 11 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 50 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazol-5-thiol obtained from 5 μL of this solution is equivalent to 8 to 12 % of that of 1-methyl-1*H*-tetrazol-5-thiol from the standard solution.

System performance: Dissolve 25 mg of Cefpiramide RS and 7 mg of cinnamic acid in the mobile phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, cinnamic acid and cefpiramide are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of 1-methyl-1*H*-tetrazol-5-thiol is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of cefpiramide.

Water Not more than 7.0 % (0.35 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefpiramide Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.06 EU/mg (potency) of cefpiramide, when Cefpiramide Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefpiramide Sodium and Cefpiramide RS, add exactly 5 mL of the internal standard solution, dissolve in the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefpiramide to that of the internal standard from the test solution and standard solution.

Amount [μg (potency)] of cefpiramide ($C_{25}H_{24}N_8O_7S_2$)

$$= \text{Amount [μg (potency)] of Cefpiramide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 4-dimethylaminoantipyrine (1 in 100)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution (pH 6.8), acetonitrile, tetrahydrofuran, and methanol (22 : 1 : 1 : 1)

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 7 minutes.

System suitability

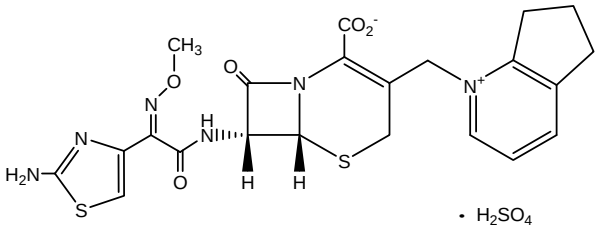
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefpiramide and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefpiramide to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5 °C.

Cefpirome Sulfate



$C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$: 612.66

Bis[(7*R*)-7-[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetamido]-3-[(6,7-dihydro-5*H*-cyclopenta[b]pyridin-1-ium-1-yl)methyl]-3,4-didehydrocepham-4-carboxylate] sulfate [*98753-19-6*]

Cefpirome Sulfate contains not less than 760 μg (potency) per mg of cefpirome ($C_{22}H_{22}N_6O_5S_2$: 514.58), calculated on the anhydrous basis.

Description Cefpirome Sulfate appears as white to pale yellowish white crystalline powder and has a slight characteristic odor.
Cefpirome Sulfate is soluble in water, and practically insoluble in ethanol (95) or in ether.
Cefpirome Sulfate is hygroscopic.

Identification (1) Dissolve 10 mg of Cefpirome Sulfate in 2 mL of water, add 3 mL of hydroxylammonium hydrochloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate, and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefpirome Sulfate in 4 mL of water, and add 1 mL of dilute hydrochloric acid while cooling in ice. To this solution add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfate while cooling in ice, allow to stand for 1 minute, and add 1 mL of *N*-1-naphthylethylenediamine dihydrochloride (1 in 1000): a purple color develops.

(3) Dissolve 5 mg of Cefpirome Sulfate in a mixture of 1 mL of ethanol (95) and 1 mL of water, add 0.1 g of 1-chloro-2,4-dinitrobenzene, and heat on a water bath for 5 minutes. After cooling, add 2 to 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-brown color develops.

(4) Determine the absorption spectra of solutions of Cefpirome Sulfate and Cefpirome Sulfate in 0.01 mol/L hydrochloric acid (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the ^{1}H spectrum of a solution of Cefpirome Sulfate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfanate as an internal reference compound: it exhibits a single signal, A, at around δ 4.1 ppm, a double signal, B, at around δ 5.9 ppm, a single signal, C, at around δ 7.1 ppm, and a multiple signal, D, at around δ 7.8 ppm. The ratio of the integrated intensity of each signal, A : B : C : D, is about 3 : 1 : 1 : 1.

(6) A solution of Cefpirome Sulfate (1 in 250) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -27 ~ -33° (0.5 g calculated on the anhydrous basis, a solution obtained by addition of water to 25 mL of acetonitrile to make 50 mL, 20 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.1 g of Cefpirome Sulfate in 10 mL of water is between 1.6 and 2.6.

Absorbance $E_{1cm}^{1\%}$ (270 nm): 405 ~ 435 (50 mg calculated on the anhydrous basis, 001 mol/L hydrochloric acid TS, 2500 mL)

Purity ***Heavy metals***—Proceed with 1.0 g of Cefpirome Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 2.5 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefpirome Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefpirome, when Cefpirome Sulfate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefpirome Sulfate and Cefpirome Sulfate RS, and dissolve each in water to make exactly 100 mL. To 5 mL each of these solutions add water to make exactly 20 mL, and use these solutions as the test solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefpirome.

Amount [μg (potency)] of cefpirome ($C_{22}H_{22}N_6O_5S_2$)
= Amount [μg (potency)] of Cefpirome Sulfate RS

$$\times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 3.45 g of ammonium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.3 using phosphoric acid. To 800 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefpirome is about 7.5 minutes.

System suitability

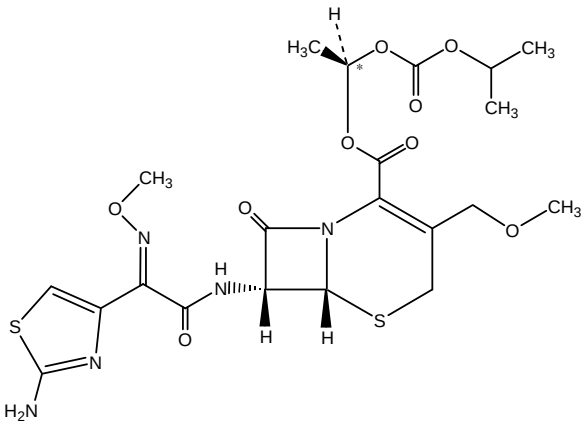
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefpirome is not less than 3600.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefpirome is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—At a temperature between 2 and 8 °C.

Cefpodoxime Proxetil



and C* epimer

$C_{21}H_{27}N_5O_9S_2$: 557.60

1-(1-Methylethyloxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(2*E*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3,4-didehydrocepham-4-carboxylate [*87239-81-4*]

Cefpodoxime Proxetil contains not less than 706 μg (potency) and not more than 774 μg (potency) per mg of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46), calculated on the anhydrous basis.

Description Cefpodoxime Proxetil appears as white or pale brown powder.
Cefpodoxime Proxetil is very soluble in acetonitrile, in methanol, or in chloroform, freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectra of solutions of Cefpodoxime Proxetil and Cefpodoxime Proxetil RS in acetonitrile (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefpodoxime Proxetil and Cefpodoxime Proxetil RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefpodoxime Proxetil in deuterated chloroform (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits double signals at around δ 1.3 ppm and at around δ 1.6 ppm, and single signals at around δ 3.3 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of each signal is 2 : 1 : 1 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +24.0 ~ +31.4° (0.1 g calculated on the anhydrous basis, acetonitrile, 20 mL, 100 mm)

Absorbance $E_{1cm}^{1\%}$ (234 nm): 324 ~ 360 (0.1 g calculated on the anhydrous basis, acetonitrile, 1000 mL)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefpodoxime Proxetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile, and acetic acid (100) (99 : 99 : 2), and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area. If necessary, compensate for changes in the baseline and the solvent peak by proceeding in the same manner with 20 μL of a mixture of water, acetonitrile, and acetic acid (100) (99 : 99 : 2). Calculate the amount of related substances by the area percentage method: the area of the peak, having the relative retention time of about 0.8 with respect to cefpodoxime proxetil isomer B, is not more than 2.0 %, the area of each peak other than cefpodoxime proxetil is not more than 1.0 %, and the total area of the peaks other than cefpodoxime proxetil is not more than 6.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water, methanol, and a solution of formic acid (1 in 50) (11 : 8 : 1)

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-65	95	5
65-145	95→15	5→85
145-155	15	85

Flow rate: Adjust the flow rate so that the retention time of cefpodoxime proxetil isomer B is about 60 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the test solution, add a mixture of water, acetonitrile, and acetic acid (100) (99 : 99 : 2) to make exactly 200 mL, and use this solution as the solution for the test for required detectability. Pipet 2 mL of the solution for the test for required detectability, and add a mixture of

water, acetonitrile, and acetic acid (100) (99 : 99 : 2) to make exactly 100 mL. Confirm that the peak areas of cefpodoxime proxetil isomers A and B obtained from 20 μL of this solution are equivalent to 1.4 to 2.6 % of the peak areas of cefpodoxime proxetil isomers A and B from the solution for the test for required detectability, respectively.

System performance: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of a mixture of water, acetonitrile, and acetic acid (100) (99 : 99 : 2). When the procedure is run with 20 μL of this solution under the above operating conditions, cefpodoxime proxetil isomer A and cefpodoxime proxetil isomer B are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of a mixture of water, acetonitrile, and acetic acid (100) (99 : 99 : 2). When the test is repeated 5 times with 20 μL each of this solution under the above operating conditions, the relative standard deviation of the peak areas of cefpodoxime proxetil isomer A and cefpodoxime proxetil isomer B is not more than 2.0 %, respectively.

Time span of measurement: About 2.5 times as long as the retention time of cefpodoxime proxetil isomer B beginning after the solvent peak.

Water Not more than 2.5 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.2 % (1 g)

Isomer Ratio Perform the test with 5 μL of the test solution obtained in the Assay as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_a and A_b, of the two isomers of cefpodoxime proxetil, having the shorter and longer retention times, respectively: $A_b/(A_a+A_b)$ is between 0.50 and 0.60.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately about 60 mg (potency) each of Cefpodoxime Proxetil and Cefpodoxime Proxetil RS, dissolve each in 80 mL of acetonitrile, add exactly 4 mL of the internal standard solution and acetonitrile to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_{T1}, Q_{T2}, Q_{S1}, and Q_{S2}, of the peak area of each isomer of cefpodoxime proxetil to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] pf cefpodoxime proxetil } (C_{15}H_{17}N_5O_6S_2) = \text{Amount [μg (potency)] pf Cefpodoxime Proxetil RS} \times \frac{Q_{T1}+Q_{T2}}{Q_{S1}+Q_{S2}}$$

Internal standard solution—Dissolve 0.3 g of ethyl parahydroxybenzoate in a solution of citric acid monohydrate in acetonitrile (1 in 2000) to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of methanol and water (9 : 11)

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 11 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ethyl parahydroxybenzoate, cefpodoxime proxetil isomer A, and cefpodoxime proxetil isomer B are eluted in this order with the resolution between the peaks of the isomers being not less than 4.0.

System repeatability: When the test is repeated 5 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefpodoxime proxetil isomer B to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefpodoxime Proxetil for Syrup

Cefpodoxime Proxetil for Syrup is a preparation for syrup, which is dissolved before use.
Cefpodoxime Proxetil for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Method of Preparation Prepare as directed under Syrups, with Cefpodoxime Proxetil.

Identification (1) Dissolve an amount of powdered Cefpodoxime Proxetil for Syrup, equivalent to 10 mg (potency) of cefpodoxime, in 2 mL of hydroxylamine

hydrochloride TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Dissolve an amount of powdered Cefpodoxime Proxetil for Syrup, equivalent to 1 mg (potency) of cefpodoxime, in 4 mL of water, add 1 mL of dilute sulfuric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate while cooling on ice, shake well, allow to stand for 1 minute, and add 1 mL of of hydrochloric acid-*N*-(1-naphthyl)-ethylenediamine hydrochloride TS: a red-purple color develops.

(3) Dissolve an amount of powdered Cefpodoxime Proxetil for Syrup, equivalent to 15 mg (potency) of cefpodoxime, in acetonitrile to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 232 nm and 236 nm.

pH The pH of a solution obtained by dissolving Cefpodoxime Proxetil for Syrup according to the label is between 4.0 and 5.5.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefpodoxime Proxetil. Weigh accurately an amount of Cefpodoxime Proxetil for Syrup, equivalent to about 0.1 g (potency) according to the labeled potency, transfer to a blender, add exactly 30 mL of the internal standard solution, blend on high speed, filter, pipet 3 mL of the filtrate, add acetonitrile to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Cefpodoxime Proxetil RS, dissolve in acetonitrile, add 15 mL of the internal standard solution and acetonitrile to make exactly 100 mL, and use this solution as the standard solution.

Internal standard solution—Dissolve 0.2 g of ethyl parahydroxybenzoate in a solution of citric acid monohydrate in acetonitrile (1 in 2000) to make 300 mL.

Containers and Storage ***Containers***—Tight containers.

Cefpodoxime Proxetil Tablets

Cefpodoxime Proxetil Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Method of Preparation Prepare as directed under Tablets, with Cefpodoxime Proxetil.

Identification (1) Dissolve an amount of powdered Cefpodoxime Proxetil Tablets, equivalent to 10 mg (potency) of cefpodoxime, in 2 mL of hydroxylamine hydrochloride TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Dissolve an amount of powdered Cefpodoxime Proxetil Tablets, equivalent to 1 mg (potency) of cefpodoxime, in 4 mL of water, add 1 mL of dilute sulfuric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 100) while cooling on ice, shake well, allow to stand for 1 minute, and add 1 mL of hydrochloric acid-*N*-(1-naphthyl)-ethylenediamine hydrochloride TS: a reddish purple color develops.

(3) Dissolve an amount of powdered Cefpodoxime Proxetil Tablets, equivalent to 15 mg (potency) of cefpodoxime, in acetonitrile to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 232 nm and 236 nm.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Cefpodoxime Proxetil Tablets at 75 revolutions per minute according to Method 2 under Dissolution Test. Previously, to a 1000 mL flask containing about 500 mL of water add 54.5 g of glycine and 42.6 g of sodium chloride, add carefully 14.2 mL of hydrochloric acid while shaking, cool, and add water to make 1000 mL. To 50 mL of this solution add water to make 900 mL, adjust the pH to 3.0 ± 0.1 with 10 mol/L sodium hydroxide if necessary, and use this solution as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately a suitable amount of Cefpodoxime Proxetil RS, dissolve in a small amount of methanol, add the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 259 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Cefpodoxime Proxetil Tablets in 30 minutes is not less than 70 % (Q).

Dissolution rate (%) with respect to the labeled amount of cefpodoxime proxetil ($C_{15}H_{17}N_5O_6S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cefpodoxime proxetil ($C_{15}H_{17}N_5O_6S$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefpodoxime Proxetil. Weigh accurately and powder not less than 20 Cefpodoxime Protexil Tablets. Weigh accurately a portion of the powder, equivalent to about 60 mg (potency) according to the labeled potency, transfer to a blender, add 40 mL of acetonitrile, blend on high speed, filter, pipet 20 mL of the filtrate, add exactly 2 mL of the internal standard solution and acetonitrile to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefprozil for Syrup

Cefprozil for Syrup is a preparation for syrup, which is suspended before use.
Cefprozil for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$: 389.43).

Method of Preparation Prepare as directed under Syrups, with Cefprozil Hydrate.

Identification (1) Dissolve separately about 50 mg (potency) each of Cefprozil for Syrup and Cefprozil (*Z*) Isomer RS in 10 mL of a mixture of acetone and 0.1 mol/L hydrochloric acid TS (4 : 1), and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid, and water (60 : 20 : 20). Expose the plate to iodine vapor: the spots obtained from the test solution and standard solution show the same R_f value.

(2) Perform the test as directed in Identification (2) under Cefprozil Hydrate.

pH The pH of a solution obtained by dissolving Cefprozil for Syrup according to the label is between 4.0 and 6.0.

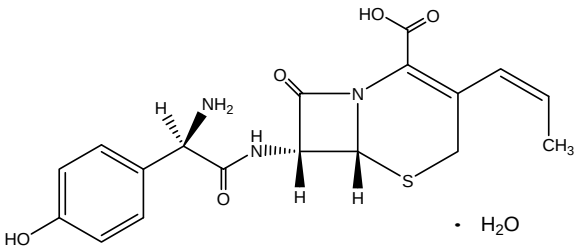
Water Not more than 3.0 % (0.2, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefprozil Hydrate. Weigh accurately an amount of Cefprozil for Syrup, equivalent to about 25 mg (potency), dissolve in water to make exactly 100 mL, centrifuge, and use the clear supernatant liquid as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefprozil Hydrate



Cefprozil $C_{18}H_{19}N_3O_5S \cdot H_2O$: 407.44

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-[(*Z*)-prop-1-enyl]-3,4-didehydrocepham-4-carboxylic acid monohydrate
[*121123-17-9*]

Cefprozil Hydrate contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg of cefprozil ($C_{18}H_{19}N_3O_5S$: 389.43), calculated on the anhydrous basis.

Description Cefprozil Hydrate appears as white to pale yellow powder.
Cefprozil Hydrate is sparingly soluble in methanol, slightly soluble in water or in dimethylsulfoxide, and practically insoluble in ethanol (95) and in acetone.

Identification (1) Determine the infrared spectra of Cefprozil Hydrate and Cefprozil (*Z*) Isomer RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Crystallinity Test It meets the requirement.

pH The pH of a solution obtained by dissolving 1 g (potency) of Cefprozil Hydrate in 200 mL of water is between 3.5 and 6.5.

Cefprozil (*E*) Isomer Proceed as directed in the Assay and perform the calculation by the following equation (the content ratio is between 0.06 and 0.11).

Content ratio of cefproxil (*E*) isomer

$$= \frac{\text{Amount } (\mu g/mg) \text{ of cefprozil } (E) \text{ isomer}}{\text{Amount } (\mu g/mg) \text{ of cefprozil } (Z) \text{ isomer} + \text{Amount } (\mu g/mg) \text{ of cefprozil } (E) \text{ isomer}}$$

Water 3.5 ~ 6.5 % (0.2 g, volumetric titration, direct titration)

Assay Weigh accurately about 25 mg (potency) of Cefprozil Hydrate, dissolve in water to make 100 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg (potency) each of Cefprozil (*Z*) Isomer RS and Cefprozil (*E*) Isomer RS, dissolve separately in water to make exactly 100 mL, pipet 5 mL each of these solutions, add water to make exactly 50 mL, and use these solutions as the standard solutions. Perform the test with 10 μL each of the test solution and standard solutions as directed under Liquid Chromatography according to the following conditions.

Amount [μg (potency)] in 1 mg of Cefprozil Hydrate = Amount (μg) of cefprozil (*Z*) isomer in 1 mg of Cefprozil Hydrate + Amount (μg) of cefprozil (*E*) isomer in 1 mg of Cefprozil Hydrate

Amount (μg) of cefprozil (*Z*) isomer in 1 mg of Cefprozil Hydrate

$$= \frac{W_1}{W} \times P_1 \times \frac{A_1}{T_1}$$

Amount (μg) of cefprozil (*E*) isomer in 1 mg of Cefprozil Hydrate

$$= \frac{W_2}{W} \times P_2 \times \frac{A_2}{T_2}$$

W: Amount (mg) of Cefprozil Hydrate taken
W_1: Amount (mg) of Cefprozil (*Z*) Isomer RS taken
A_1: Peak area of cefprozil (*Z*) isomer from the test solution
T_1: Peak area of cefprozil (*Z*) isomer from the standard solution
P_1: Potency (μg/mg) of Cefprozil (*Z*) Isomer RS
W_2: Amount (mg) of Cefprozil (*E*) Isomer RS taken
A_2: Peak area of cefprozil (*E*) isomer from the test solution
T_2: Peak area of cefprozil (*E*) isomer from the standard solution
P_2: Potency (μg/mg) of Cefprozil (*E*) Isomer RS

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 280 nm)
Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Mobile phase: Dissolve 20.7 g of ammonium dihydrogen phosphate in 1800 mL of water, adjust the pH to 4.4 with phosphoric acid, and add 200 mL of acetonitrile.
Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Cefprozil Tablets

Cefprozil Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$: 389.43).

Method of Preparation Prepare as directed under Tablets, with Cefprozil Hydrate.

Identification Powder Cefprozil Tablets, and proceed as directed in the Identification under Cefprozil for Syrup.

Water Not more than 7.0 % (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Cefprozil Tablets at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of water as the dissolution solution. Take an amount of the dissolved solution 45 minutes after the start of the test, filter through a membrane filter with a pore size not exceeding 0.5 μm, add water so that each mL contains 0.4 mg (potency), and use this solution as the test solution. Proceed as directed in the operating conditions in the Assay, and calculate the dissolution rate. The dissolution rate of Cefprozil Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefprozil Hydrate. Weigh accurately and powder not less than 20 Cefprozil Tablets. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency), add water and shake well to make exactly 100 mL, centrifuge, and use the clear supernatant liquid as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefradine Capsules

Cefradine Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefradine ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Capsules, with Cefradine Hydrate.

Identification Weigh an amount of Cefradine Capsules, equivalent to about 0.25 g (potency) according to the labeled potency, place in a stoppered test tube, add 10 mL of water and 5 mL of hydrochloric acid, mix, boil in a water bath for 10 minutes, cool, and centrifuge: an orange color is produced.

Loss on Drying Not more than 7.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours)

Dissolution Test Perform the test with 1 capsule of Cefradine Capsules at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of 0.12 mol/L hydrochloric acid TS as the dissolution solution. Take the dissolved solution 45 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Cefradine RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Cefradine Capsules in 45 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of cefradine ($C_{16}H_{19}N_3O_4S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cefradine ($C_{16}H_{19}N_3O_4S$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefradine Hydrate. Weigh accurately the contents of not less than 20 Cefradine Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefradine for Injection

Cefradine for Injection is a preparation for injection, which is dissolved before use.
Cefradine for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefradine ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directe under Injections, with Cefradine.

Description Cefradine for Injection appears as white to pale yellowish white powder.

Identification Perform the test as directed in the Identification under Cefradine Capsules.

pH The pH of a solution obtained by dissolving an amount of Cefradine for Injection, equivalent to 1 g (potency) of cefradine, in 100 mL of water is between 8.0 and 9.6.

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of cefradine.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefradine Hydrate. Weigh accurately an amount of Cefradine for Injection, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Cefradine for Syrup

Cefradine for Syrup is a preparation for syrup, which is dissolved before use.
Cefradine for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefradine ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Syrups, with Cefradine Hydrate.

Identification To 2 g of Cefradine for Syrup add 10 mL of ether, mix well, and filter. To the residue add 10 mL of ethanol (99.5), mix well, and filter. Transfer the residue to a glass-stoppered flask, add 2 mL of water and 1 mL of hydrochloric acid, mix, boil in a water bath for 10 minutes, cool, and centrifuge: the color of the water layer changes through yellow-green to orange.

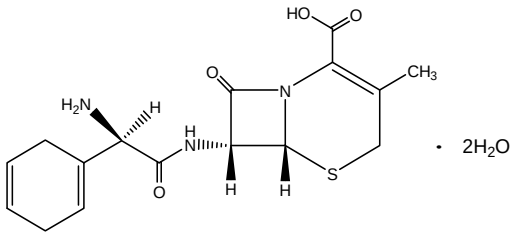
Water Not more than 1.5 % (0.3 g, volumetric titration, direct titration)

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefradine Hydrate. Weigh accurately an amount of Cefradine for Syrup, equivalent to about 50 mg (potency) according to the labeled potency, add the mobile phase, shake, make exactly 100 mL, filter or centrifuge if necessary, and use this solion as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefradine Hydrate



$C_{16}H_{19}N_3O_4S \cdot 2H_2O$: 385.44

(6*R*,7*R*)-7-[2-Amino-2-(cyclohexa-1,4-dien-1-yl)aceamido]-3-methyl-3,4-didehydrocepham-4-carboxylic acid monohydrate [*31828-50-9*]

Cefradine Hydrate contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg of cefradine ($C_{16}H_{19}N_3O_4S$: 349.41), calculated on the anhydrous basis.

Description Cefradine Hydrate is a white to pale yellowish white crystalline powder, is odorless or has a little bit of a characteristic odor and bitter taste.
Cefradine Hydrate is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in ether.

Identification Determine the infrared spectra of Cefradine Hydrate and Cefradine RS, as directed in the potassium bromide disk method under the Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve each of Cefradine Hydrate and Cefradine Hydrate RS in methanol, evaporate to dryness, and repeat the test on the residues.

Crystallinity Test It meets the requirement.

pH The pH of a solution obtained by dissolving 0.1 g of Cefradine Hydrate in 10 mL of water is between 3.5 and 6.0.

Purity (1) ***Heavy metals***— Proceed with 2.0 g of Cefradine Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***— Prepare the test solution with 2.0 g of Cefradine Hydrate according to Method 4 and perform the test (not more than 1 ppm).

(3) ***Cefalexin***—Proceed as directed in the Assay, and calculate the amount of cefalexin according to the following equation (not more than 5.0 %).

$$\text{Amount (\%) of cefalexin } (C_{16}H_{17}N_3O_4S) = \frac{A_T}{A_S} \times 100$$

A_T: Peak area of cefalexin obtained from the test solution

A_S: Total area of the peaks of cefradine and cefalexin obtained from the test solution

Water 8.5 ~ 10.5 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefradine Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.20 EU/mg of cefradine, when Cefradine Hydrate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cefradine Hydrate and Cefradine RS, dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the amount of cefradine by the following equation.

$$\text{Amount [μg (potency)] of cefradine } (C_{16}H_{19}N_3O_4S) = \text{Amount [μg (potency)] of Cefradine RS} \times \frac{A_T}{A_S}$$

A_T: Total area of the peaks of cefradine and cefalexin obtained from the test solution

A_S: Total area of the peaks of cefradine and cefalexin obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: A mixture of water, methanol, 0.5 mol/L sodium acetate, and 0.7 mol/L acetic acid (782 : 200 : 15 : 3)

Flow rate: 1.0 mL per minute.

System suitability

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the relative retention times of cefalexin and cefradine are 0.8 and 1.0, respectively, with the resolution between these peaks being not less than 2.0.

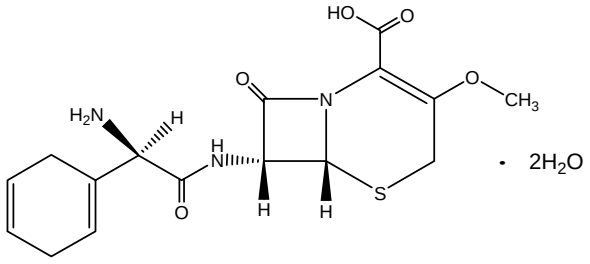
System repeatability: When the test is repeated 5 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of cefradine is not more than 2.0 %.

System suitability solution—Dissolve a suitable amount each of Cefradine RS and Cefalexin RS in the mobile phase to make a solution so that each mL contains 0.5 mg.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefroxadine Hydrate



Cefroxadine $C_{16}H_{19}N_3O_5S \cdot 2H_2O$: 401.43

(6*R*,7*R*)-7-[2-Amino-2-(cyclohexa-1,4-dien-1-yl)aceamido]-3-methyloxy-3,4-didehydrocepham-4-carboxylic acid monohydrate [*95615-72-8*]

Cefroxadine Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg of cefroxadine ($C_{16}H_{19}N_3O_5S$: 365.40), calculated on the anhydrous basis.

Description Cefroxadine Hydrate appears as pale yellowish white to pale yellow crystalline particles or powder.

Cefroxadine Hydrate is very soluble in formic acid, slightly soluble in water or in methanol, and very slightly soluble in acetonitrile or in ethanol (95).

Cefroxadine Hydrate dissolves in 0.001 mol/L hydrochloric acid TS or in dilute acetic acid.

Identification (1) Determine the absorption spectrum of solutions of Cefroxadine Hydrate and Cefroxadine RS in 0.001 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefroxadine Hydrate in deuterated formic acid for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three sharp single signals, A, B, and C, at around δ 2.8 ppm, at around δ 4.1 ppm, and at around δ 6.3 ppm. The ratio of the integrated intensity of each signal, A : B : C, is about 4 : 3 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +95 ~ +108° (0.1 g calculated on the anhydrous basis, diluted acetic acid (100) (3 in 25), 100 mL, 100 mm).

Purity (1) ***Heavy metals***—Weigh 1.0 g of Cefroxadine Hydrate in a porcelain crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, and carbonize by gently heating. After cooling, add 2 mL of nitric acid, heat carefully, and ignite to incinerate at 500 to 600 °C. If a carbonized substance remains, moisten with a small amunt of nitric acid, and ignite to incinerate. After cooling, add 6 mL of hydrochloric acid, and evaporate to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm in a water bath to dissolve. After cooling, adjust the pH to between 3 and 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, transfer to a Nessler tube, wash the porcelain crucible with 10 mL of water, and add the washings and water to the tube to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: put 2.0 mL of standard lead solution and 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) in a porcelain crucible, and proceed as directed for the preparation of the test solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Cefroxadine Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 10 mg of Cefroxadine Hydrate in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly

100 mL, and use this solution as the standard solution. Perform the test with 40 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the areas of the peaks having the relative retention times of about 0.07, 0.6, and 0.8 with respect to cefroxadine from the test solution are not larger than 2 times, 4 times, and 1 times the peak area of cefroxadine from the standard solution, respectively. The area of each peak other than the above peaks and cefroxadine from the test solution is not larger than 1/2 times the peak area of cefroxadine from the standard solution, and the total area of the peaks other than cefroxadine from the test solution is not larger than 6 times the peak area of cefroxadine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 1.4 g of sodium perchlorate in 1000 mL of a mixture of water and acetonitrile (489 : 11).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 20 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefroxadine obtained from 40 μL of this solution is equivalent to 7 to 13 % of the peak area of cefroxadine from the standard solution.

System performance: Dissolve 3 mg of Cefroxadine Hydrate and 15 mg of orcin in 100 mL of the mobile phase. When the procedure is run with 40 μL of this solution under the above operating conditions, orcin and cefroxadine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 40 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefroxadine is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of cefroxadine.

Water 8.5 ~ 12.0 % (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 50 mg (potency) each of Cefroxadine Hydrate and Cefroxadine RS, dissolve each in a mixture of dilute acetic acid and phosphoric acid (500 : 1), add exactly 5 mL of the internal standard solution and a mixture of dilute acetic acid and phosphoric acid (500 : 1) to make 200 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefroxadine to that of the internal standard.

$$\text{Amount [μg (potency)] of cefroxadine } (C_{16}H_{19}N_3O_5S) = \text{Amount [μg (potency)] of Cefroxadine RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500 : 1) to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 50) and acetonitrile (97 : 3)

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 10 minutes.

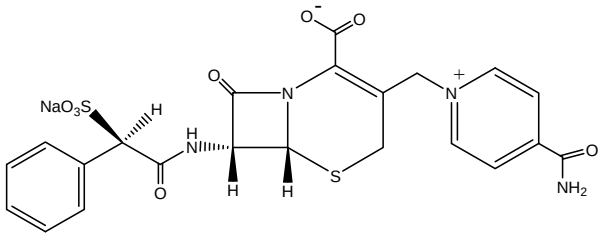
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefroxadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefroxadine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefsulodin Sodium



$C_{22}H_{19}N_4NaO_8S_2$: 554.53

Sodium (6*R*,7*R*)-3-[(4-carbamoylpyridin-1-ium-1-yl)methyl]-7-[(2R)-2-phenyl-2-sulfonatoacetamido]-3,4-didehydrocepham-4-carboxylate [*52152-93-9*]

Cefsulodin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg of cefsulodin ($C_{22}H_{20}N_4O_8S_2$: 532.55), calculated on the anhydrous basis.

Description Cefsulodin Sodium appears as white to pale yellow crystals or crystalline powder.
Cefsulodin Sodium is freely soluble in water or in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).
Cefsulodin Sodium is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Cefsulodin Sodium and Cefsulodin Sodium RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefsulodin Sodium and Cefsulodin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefsulodin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal, A, between δ 7.3 and δ 7.7 ppm, and double signals, B and C, at around δ 8.4 ppm and at around δ 9.1 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C, is about 5 : 2 : 2.

(4) Cefsulodin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +16.5 ~ +20.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Cefsulodin Sodium in 10 mL of water is between 3.3 and 4.8.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the solution is clear.

(2) ***Heavy metals***—To 1.0 g of Cefsulodin Sodium add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), mix, fire the ethanol to burn, then heat gradually to incinerate. After cooling, add 2 mL of nitric acid, heat carefully, then ignite at 500 to 600 °C to incinerate. If a carbonized residue still remains, add a small amount of nitric acid, and ignite again to incinerate. After cooling, add 6 mL of hydrochloric acid to the residue, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm on a water bath to dissolve. Adjust the pH to between 3 and 4 with ammonia TS, and add 2 mL of dilute acetic acid. Filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control wolution as follows: to 2.0 mL of standard lead solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), and fire the ethanol to burn. After cooling, add 2 mL of nitric acid, heat carefully, then ignite at 500 to 600 °C. After cooling, add 6 mL of hydrochloric acid to the residue, then proceed in the same manner as for the preparation of the test solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefsulodin Sodium according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) and 15 mL of dilute hydrochloric acid instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50) and 3 mL of hydrochloric acid (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately 0.10 g of Cefsulodin Sodium, dissolve in water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of isonicotinic acid amide and about 20 mg of Cefsulodin Sodium RS (separately determine the water in the same manner as Cefsulodin Sodium), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of related substances by the following equations: isonicotinic acid amide is not more than 1.0 %, and the total amount of other related substances is not more than 1.2 %.

$$\text{Amount (\%) of isonicotinic acid amide} = \frac{A}{B_I} \times \frac{W_I}{W_T} \times 5$$

$$\text{Total amount (\%) of other related substances} = \frac{B}{B_S} \times \frac{W_S}{W_T} \times 5$$

A: Peak area of isonicotinic acid amide from the test solution

B: Total area of the peaks other than cefsulodin and isonicotinic acid amide from the test solution

B_I: Peak area of isonicotinic acid amide from the standard solution

B_S: Peak area of cefsulodin from the standard solution
W_T: Amount (g) of Cefsulodin Sodium taken
W_S: Amount (g) of Cefsulodin Sodium RS taken
W_I: Amount (g) of isonicotinic acid amide taken

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Change the mobile phase A to the mobile phase B at 14 minutes after injection of the test solution.

Mobile phase A: A mixture of a solution of ammonium sulfate solution (1 in 100) and acetonitrile (97 : 3)

Mobile phase B: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (92 : 8)

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak areas of isonicotinic acid amide and cefsulodin obtained from 10 μL of this solution are equivalent to 7 to 13 % of the peak areas of isonicotinic acid amide and cefsulodin from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of cefsulodin

Water Not more than 5.0 % (1 g, volumetric titration, direct titration. Avoid moisture absorption when taking the sample, and use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination.)

Sterility Test It meets the requirement, when Cefsulodin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.125 EU/mg (potency) of cefsulodin, when Cefsulodin Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Cefsulodin Sodium and Cefsulodin Sodium RS, dissolve each in water to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak ares, A_T and A_S, of cefsulodin.

$$\text{Amount [μg (potency)] of cefsulodin } (C_{22}H_{20}N_4O_8S_2) = \text{Amount [μg (potency)] of Cefsulodin Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm inlength, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97 :3)

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

System suitability

System performance: Dissolve 40 mg of isonicotinic acid amide in 25 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Ceftazidime for Injection

Ceftazidime for Injection is a preparation for injection, which is dissolved before use.
Ceftazidime for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of ceftzidime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Method of Preparation Prepare as directed under Injections, with Ceftazidime Hydrate.

Description Ceftazidime for Injection appears as white to pale yellowish white powder.

Identification Determine the absorption spectrum of a solution of Ceftazidime for Injection in phosphate

buffer solution (pH 6.0) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 255 nm and 259 nm.

pH The pH of a solution obtained by dissolving an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of ceftazidime, in 10 mL of water is between 5.8 and 7.8.

Purity ***Clarity of solution***—Dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of ceftazidime hydrate according to the labeled amount, in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL: the solution is clear, and the absorbance of this solution, determined at 420 nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.3.

Loss on Drying Not more than 14.0 % (0.1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement

Bacterial Endotoxins Less than 0.067 EU/mg (potency) of ceftazidime.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

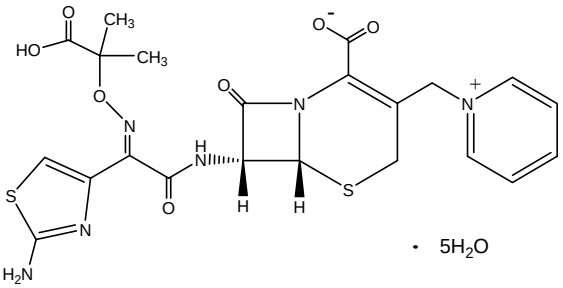
Assay Weigh accurately the mass of the contents of not less than 10 containers of Ceftazidime for Injection. Weigh accurately an amount of the contents, equivalent to about 0.25 g (potency) of ceftazidime hydrate, and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg (potency) of Ceftazidime RS, and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ceftazidime Hydrate.

$$\text{Amount [mg (potency)] of ceftazidime } (C_{22}H_{22}N_6O_7S_2) = \text{Amount [mg (potency)] of Ceftazidime RS} \times \frac{Q_T}{Q_S} \times 10$$

Internal standard solution—A solution of dimedone in 0.05 mol/L phosphate buffer solution (pH 7.0) (11 in 10000)

Containers and Storage ***Containers***—Hermetic containers.
Storage—Light-resistant.

Ceftazidime Hydrate



$C_{22}H_{22}N_6O_7S_2 \cdot 5H_2O$: 636.65

(6*R*,7*R*)-7-[(2*E*)-2-(2-Amino-1,3-thiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetamido]-3-(pyridin-1-ium-1-yl)methyl-3,4-didehydrocepham-4-carboxylate pentahydrate [*78439-06-2*]

Ceftazidime Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of ceftazidime ($C_{22}H_{22}N_6O_7S_2$: 546.58), calculated on the dried basis.

Description Ceftazidime Hydrate is a white to pale yellowish white crystalline powder.
Ceftazidime Hydrate is slightly soluble in water, and very slightly soluble in acetonitrile or in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Ceftazidime Hydrate and Ceftazidime RS in pH 6.0 phosphate buffer solution (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ceftazidime Hydrate and Ceftazidime RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 50 mg of Ceftazidime Hydrate add 5 mg of dried sodium carbonate, dissolve in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 1.5 ppm and at around δ 6.9 ppm, respectively, and a multiple signal, C, between δ 7.9 and 9.2

ppm. The ratio of the integrated intensity of each signal, A : B : C, is about 6 : 1 : 5.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -28 ~ -34° (0.5 g calculated on the dried basis, phosphate buffer solution (pH 6.0), 100 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.5 g of Ceftazidime Hydrate in 100 mL of water is between 3.0 and 4.0.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Ceftazidime Hydrate in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL: the solution is clear, and the absorbance of this solution, determined at 420 nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.20.

(2) ***Free pyridine***—Weigh accurately about 50 mg of Ceftazidime Hydrate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately 0.1 g of pyridine, dissolve in the mobile phase to make exactly 100 mL, pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of pyridine in each solution: the amount of free pyridine is not more than 0.3 %.

$$\text{Amount (mg) of free pyridine} = \text{Amount (mg) of pyridine taken} \times \frac{H_T}{H_S} \times \frac{1}{1000}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 2.88 g of ammonium dihydrogen phosphate in 500 mL of water, add 300 mL of acetonitrile and water to make 1 L, and adjust the pH to 7.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 4 minutes.

System suitability

Test for required detectability: Confirm that the peak height of pyridine obtained from 10 μL of the standard solution is equivalent to about 50 % of the full scale.

System performance: Dissolve 5 mg of Ceftazidime Hydrate in 100 mL of a solution of pyridine in the mobile phase (1 in 20000). When the procedure is run with 10 μL of this solution under the above operating conditions, ceftazidime and pyridine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of pyridine is not more than 5.0 %.

(3) ***Heavy metals***—Proceed with 1.0 g of Ceftazidime Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Proceed with 1.0 g of Ceftazidime Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) ***Related substances (trityl-t-butyl substance and t-butyl substance)*** —Dissolve 0.1 g of Ceftazidime Hydrate in 2 mL of diluted disodium hydrogen phosphate TS (1 in 3), and use this solution as the test solution. Pipet 1 mL of this solution, add diluted disodium hydrogen phosphate TS (1 in 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of *n*-butyl acetate, acetic acid (100), acetate buffer solution (pH 4.5), and 1-butanol (16 : 16 : 13 : 3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots which appear upper in position than the principal spot from the test solution are not more intense than the spot from the standard solution.

(6) ***Other related substances***—Dissolve 20 mg of Ceftazidime Hydrate in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, dissolve in the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than ceftazidime from the test solution is not larger than the peak area of ceftazidime from the standard solution, and the total area of the peaks other than ceftazidime from the test solution is not larger than 5 times the peak area of ceftazidime from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.0 g of ammonium dihydrogen phosphate in 750 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 870 mL. To this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftazidime is about 4 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 5 mL. Confirm that the peak area of ceftazidime obtained from 5 μL of this solution is equivalent to 15 to 25 % of that of ceftazidime from 5 μL of the standard solution.

System performance: Dissolve 10 mg each of Ceftazidime Hydrate and acetanilide in 20 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, ceftazidime and acetanilide are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ceftazidime is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of ceftazidime beginning after the solvent peak.

Loss on Drying 13.0 ~ 15.0 % (0.1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement, when Ceftazidime Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg of ceftazidime, when Ceftazidime Hydrate is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Ceftazidime Hydrate and Ceftazidime RS, dissolve each in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution and 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftazidime to that of the internal standard.

Amount [μg (potency)] of ceftazidime ($C_{22}H_{22}N_6O_7S_2$)

$$= \text{Amount [μg (potency)] of Ceftazidime RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dimedone in 0.05 mol/L phosphate buffer solution (pH 7.0) (11 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with hexasilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 4.26 g of anhydrous disodium hydrogen phosphate and 2.72 g of potassium dihydrogen phosphate in 980 mL of water, and add 20 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftadizime is about 4 minutes.

System suitability

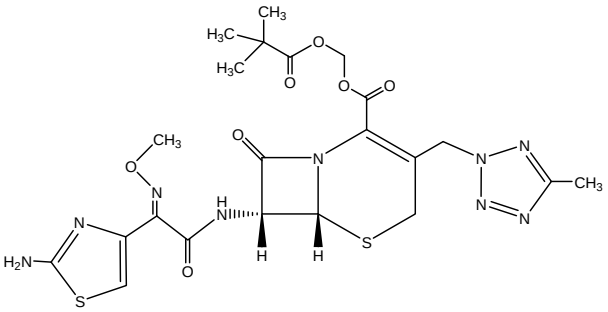
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and ceftazidime are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftazidime to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—tight containers.

Storage—Light-resistant.

Cefteram Pivoxil



$C_{22}H_{27}N_9O_7S_2$: 593.64

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(2*E*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetamido]-3-[(5-methyltetrazol-2-yl)methyl]-3,4-didehydrocepham-4-carboxylate [*82547-81-7*]

Cefteram Pivoxil contains not less than 743 μg (potency) per mg of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49), calculated on the anhydrous basis.

Description Cefteram Pivoxil appears as white to pale yellowish white powder.
Cefteram Pivoxil is very soluble in acetonitrile, freely soluble in methanol, in ethanol (95), or in chloroform, and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Cefteram Pivoxil and Cefteram Pivoxil RS in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, and C, at around δ 1.2 ppm, at around δ2.5 ppm, and at around δ 4.0 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C, is about 3 : 1 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +35 ~ +43° (0.4 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cefteram Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Cefteram Pivoxil according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50 mg of Cefteram Pivoxil in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil, is not larger than 1.25 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, is not larger than 0.25 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil from the test solution is not larger than 2.75 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, after multiplying by its relative response factor, 0.74.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7 to 13 % of that of cefteram pivoxil from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefteram pivoxil is not more than 3.0 %.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.

Water Not more than 3.0 % (0.3 g, coulometric titration)

Assay Weigh accurately about 40 mg (potency) each of Cefteram Pivoxil and Cefteram Pivoxil Mesitylene Sulfonate RS, dissolve each in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefteram pivoxil to that of the internal standard.

$$\text{Amount [μg (potency)] of cefteram } (C_{16}H_{17}N_9O_5S_2) = \text{Amount [μg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl parahydroxybenzonate in diluted acetonitrile (1 in 2) (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 100 mL of acetic acid (pH 5.0)·sodium acetate buffer solution and 375 mL of acetonitrile add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefteram pivoxil is about 14 minutes.

System suitability

System performance: When the procedure is run with 10 μL each of the standard solution under the above operating conditions, the internal standard and cefteram pivoxil are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefteram pivoxil to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—In a cold place.

Cefteram Pivoxil Fine Granules

Cefeteram Pivoxil Fine Granules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Method of Preparation Prepare into fine granules as directed under Powders, with Cefteram Pivoxil.

Identification To a portion of powdered Ceteram Pivoxil Fine Granules, equivalent to 0.1 g (potency) of cefteram pivoxil according to the labeled amount, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 262 nm and 266 nm.

Purity ***Related substances***—Powder Cefteram Pivoxil Fine Granules if necessary. To a portion, equivalent to 0.1 g (potency) of cefteram pivoxil according to the labeled amount, add diluted acetonitrile (1 in 2) to make 100 mL. Disperse the particles with the aid of ultrasonic waves, filter, and use the filtrate as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil from the test solution, is not larger than 1.75 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil from the test solution, is not larger than 0.68 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil from the test solution is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. Use the area of the peak, having the relative retention time of bout 0.1 with respect to cefteram pivoxil, after multiplying by its relative response factor, 0.74.

Operating conditions

Proceed as directed in the operating conditions in the Purity (3) under Cefteram Pivoxil.

System suitability

Proceed as directed in the system suitability in the Purity (3) under Cefteram Pivoxil.

Water Not more than 0.3 % (0.1 g (potency), coulometric titration)

Particle Size Distribution Test It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Powder Cefteram Pivoxil Fine Granules if necessary. Weigh accurately a portion, equivalent to about 0.3 g (potency) of cefteram pivoxil according to the labeled amount, and add exactly 30 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 300 mL. Disperse the particles with the aid of ultrasonic waves, filter, and use the filtrate as the test solution. Separately, weigh accurately about 50 mg (potency) of Cefteram Pivoxil Mesitylene Sulfonate RS, dissolve in diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefteram pivoxil to that of the internal standard.

$$\text{Amount [mg (potency)] of cefteram } (C_{16}H_{17}N_9O_5S_2) = \text{Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS} \times \frac{Q_T}{Q_S} \times 6$$

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000)

Operating conditions

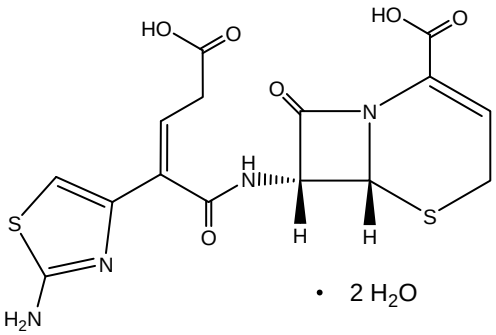
Proceed as directed in the operating conditions in the Assay under Cefteram Pivoxil.

System suitability

Proceed as directed in the system suitability in the Assay under Cefteram Pivoxil.

Containers and Storage ***Containers***—Tight containers.

Ceftibuten Hydrate



Ceftibuten $C_{15}H_{14}N_4O_6S_2 \cdot 2H_2O$: 446.46

(6*R*,7*R*)-7-[(2*Z*)-2-(2-Aminothiazol-4-yl)-4-carboxybut-2-enamido]-3,4-didehydrocepham-4-carboxylic acid dihydrate [*118081-34-8*]

Ceftibuten Hydrate contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg of ceftibuten ($C_{15}H_{14}N_4O_6S_2$: 410.42), calculated on the anhydrous basis.

Description Ceftibuten Hydrate appears as white to pale yellowish white crystalline powder.
Ceftibuten Hydrate is freely soluble in *N,N*-dimethylformamide or in dimethylsulfoxide, and practically insoluble in water, in ethanol (95), or in ether.

Identification (1) Determine the absorption spectrum of a solution of Ceftibuten Hydrate in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 265 nm.

(2) Determine the infrared spectrum of Ceftibuten Hydrate as directed in the paste method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3249 cm^{-1}, 1772 cm^{-1}, 1700 cm^{-1}, 1651 cm^{-1}, and 1544 cm^{-1}.

(3) Determine the ^1H spectrum of a solution of Ceftibuten Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 30) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits double signals, A and B, at around δ 3.2 ppm and at around δ 5.1 ppm, a quartet signal, C, at around δ 5.8 ppm, and a single signal, D, at around δ 6.3. The ratio of the integrated intensity of each signal except the signal at around δ 3.2 ppm, B : C : D, is about 1 : 1 : 1.

Specific Optical Rotation $[\alpha]_D^{20}$: +135 ~ +155° (0.3 g calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0), 50 mL, 100 mm)

Absorbance $E_{1cm}^{1\%}$ (263 nm): 320 ~ 345 (20 mg calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0), 1000 mL).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Ceftibuten Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 25 mg (potency) of Ceftibuten Hydrate, dissolve in 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 20 mL, pipet 4.0 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 20 mL, and use this solution as the test solution. Pipet 5 mL of the test solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make 100 mL, and use this solution as the standard solution. Keep the test solution and standard solution at a temperature not exceeding 5 °C, and use within 2 hours. Perform the test with exactly 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of the peak other than ceftibuten from the test solution is not larger than 1/5 times the peak area of ceftibuten from the standard solution, and the total area of the peaks other than ceftibuten from the test solution is not larger than the peak area of ceftibuten from the standard solution (total related substances is not more than 5.0 %).

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.

System suitability

Detection sensitivity: Pipet 2 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make 20 mL. Confirm that the peak area of ceftibuten obtained from 5 μL of this solution is equivalent to 7 to 13 % of that of ceftibuten from the standard solution.

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 20 mL of 1 mol/L hydrochloric acid TS, and allow to stand at 40 °C for 1 hour. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the isomer and ceftibuten are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ceftibuten is not more than 2.0 %.

Water 8.0 ~ 13.0 % (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for water determination (5 : 1) instead of methanol for water determination.)

Residue on Ignition Not more than 0.1 % (1g)

Assay Weigh accurately about 10 mg (potency) each of Ceftibuten Hydrate and Ceftibuten Hydrochloride RS, dissolve each in about 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0), add exactly 4 mL of the internal standard solution, shake, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftibuten to that of the internal standard. Keep the test solution and standard solution at a temperature not exceeding 5 °C, and use within 2 hours.

Amount [μg (potency)] of ceftibuten ($C_{15}H_{14}N_4O_6S_2$)
= Amount [μg (potency)] of Ceftibuten Hydrochloride RS $\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (3 in 4000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 263 nm)

Column: A stainless steel column about 4 mm in internal diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.005 mol/L *n*-decyl trimethylammonium bromide TS and acetonitrile (4 : 1)

Flow rate: Adjust the flow rate so that the retention time of ceftibuten is about 10 minutes.

System suitability

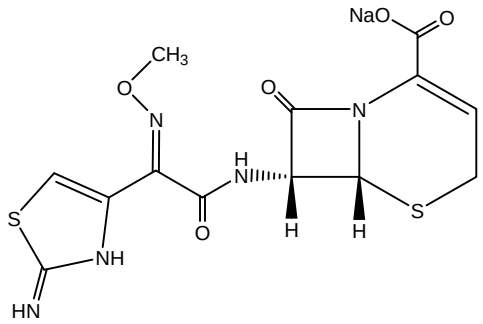
System performance: Dissolve 5 mg of Ceftibuten Hydrate in 50 mL of 1 mol/L hydrochloric acid TS, and allow to stand at room temperature for 4 hours. To 10 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the isomer and ceftibuten are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftibuten to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 5 °C.

Ceftizoxime Sodium



$C_{13}H_{12}N_5NaO_5S_2$: 405.38

Sodium (6*R*,7*R*)-7-[(2*E*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3,4-didehydrocepham-4-carboxylate [*68401-82-1*]

Ceftizoxime Sodium contains not less than 925 μg (potency) and not more than 965 μg (potency) per mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$: 383.40), calculated on the anhydrous basis.

Description Ceftizoxime Sodium appears as white to pale yellow crystals or crystalline powder.
Ceftizoxime Sodium is very soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Ceftizoxime Sodium (1 in 63000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 233 nm and 237 nm.

(2) Determine the infrared spectra of Ceftizoxime Sodium and Ceftizoxime Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Ceftizoxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits a single signal at around δ 4.0 ppm, a multiple signal at around δ 6.3 ppm, and a single signal at around δ 7.0 ppm. The ratio of the integrated intensity of each signal is 3 : 1 : 1.

(4) Ceftizoxime Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +125 ~ +145° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1 g (potency) of Ceftizoxime Sodium in 10 mL of water is between 6.0 and 8.0.

Absorbance $E_{1cm}^{1\%}$ (235 nm): 410 ~ 450 (1.6 mg calculated on the anhydrous basis, water, 100 mL)

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Ceftizoxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Ceftizoxime Sodium according to Method 3, and perform the test (not more than 1 ppm).

(4) ***Related substances***—Dissolve 0.11 g of Ceftizoxime Sodium in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the test solution by the automatic integration method: the area of the peak other than ceftizoxime is not more than 0.5 % of the peak area of ceftizoxime, and the total area of the peaks other than ceftizoxime is not more than 1.0 % of the peak area of ceftizoxime.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 2.31 g of disodium hydrogen phosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjust the pH to 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 200 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftizoxime is about 12 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add 0.1 mol/L phosphate buffer solution (pH 7.6) to make exactly 100 mL, and use this solution as the solution for the test for required detectability. To exactly 1 mL of the solution for the test for required detectability add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Confirm that the peak area of ceftizoxime obtained from 5 μL of this solution is equivalent to 7 to 13 % of that of ceftizoxime from the solution for the test for required detectability.

System performance: Dissolve about 10 mg of Ceftizoxime RS in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the system suitability solution. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ceftizoxime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of ceftizoxime is not more than 2.0 %.

Time span of measurement: About 5 times as long as the retention time of ceftizoxime beginning after the solvent peak.

Water Not more than 8.5 % (0.4 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Ceftizoxime Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of ceftizoxime, when Ceftizoxime Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Ceftizoxime Sodium and Ceftizoxime RS, dissolve each in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL, and use these solutions as the test stock solution and standard stock solution, respectively. Pipet 2 mL each of the test stock solution and standard stock solution, add 10.0 mL of the internal standard solution and 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftizoxime to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of ceftizoxime } (C_{13}H_{13}N_5O_5S_2) = \text{Amount [μg (potency)] of Ceftizoxime RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *m*-hydroxybenzoic acid in 0.1 mol/L phosphate buffer solution (pH 7.0) (3 in 500)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 2.31 g of disodium hydrogen phosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjut the pH to 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 450 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftizoxime is about 4 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ceftizoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0. The symmetry factor of each peak is not more than 2.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftizoxime to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ceftizoxime Sodium for Injection

Ceftizoxime Sodium for Injection is a preparation for injection, which is dissolved before use.
Ceftizoxime Sodium contains not less than 90.0 % and not more than 120.0 % of the labeled amount of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$: 383.41).

Method of Preparation Prepare as directed under Injections, with Ceftizoxime Sodium.

Description Ceftizoxime Sodium for Injection appears as white to pale yellow powder.

Identification (1) Dissolve an amount of Ceftizoxime Sodium for Injection, equivalent to 10 mg (potency) of ceftizoxime, in 2 mL of water, add 3 mL of hydroxylamine hydrochloride TS, allow to stand for 5 minutes, and add 1 mL of acidic ammonium iron (III) sulfate TS: a red-brown color develops.

(2) Dissolve an amount of Ceftizoxime for Injection, equivalent to 1 mg (potency) of ceftizoxime, in 4 mL of water, add 1 mL of dilute hydrochloric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfate TS while cooling on ice, allow to stand for 1 minute, and add 1 mL of a solution of *N*-(1-naphthyl)ethylenediamine hydrochloride (1 in 1000): a purple color develops.

(3) Proceed as directed in the Identification (1) under Ceftizoxime Sodium.

pH The pH of a solution obtained by dissolving an amount of Ceftizoxime Sodium for Injection, equivalent to 1.0 g (potency) of ceftizoxime, in 10 mL of water is between 6.0 and 8.0.

Water Not more than 8.5 % (0.1 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of ceftizoxime.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Ceftizoxime Sodium. Weigh accurately an amount of Ceftizoxime Sodium for Injection, equivalent to about 0.5 g (potency) according to the labeled potency, and dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) so that each mL contains about 5 mg (potency). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Ceftriaxone Sodium for Injection

Ceftriaxone Sodium for Injection is a preparation for injection, which is dissolved before use.
Ceftriaxone Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$: 554.58).

Method of Preparation Prepare as directed under Injections, with Ceftriaxone Sodium Hydrate.

Description Ceftriaxone Sodium for Injection appears as white to yellowish white powder.

Identification Determine the infrared spectra of Ceftriaxone Sodium for Injection and Ceftriaxone Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH The pH of a solution obtained by dissolving an amount of Ceftriaxone Sodium for Injection, equivalent to 1.2 g (potency) of ceftriaxone, in 10 mL of water is between 6.0 and 8.0.

Water 8.0 ~ 11.0 % (0.1 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of ceftriaxone.

Foreign Insoluble Matter Test It meets the requirement.

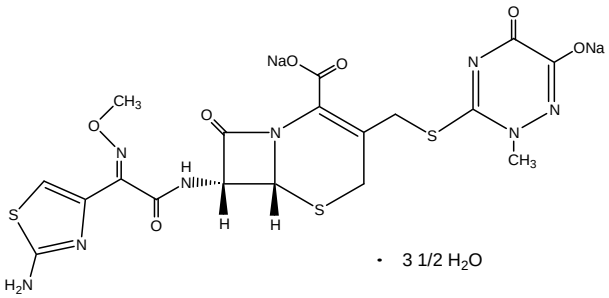
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Ceftriaxone Sodium Hydrate. Weigh accurately an amount of Ceftriaxone Sodium for Injection, equivalent to about 0.1 g (potency) according to the labeled potency, and dissolve in a mixture of water and acetonitrile (11 : 9) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile (11 : 9) to make exactly 200 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Ceftriaxone Sodium Hydrate



$C_{18}H_{16}N_8Na_2O_7S_3 \cdot 3½H_2O$: 661.60

Disodium (6*R*,7*R*)-7-[(2*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(2,5-dihydro-2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-ylsulfanylmethyl)-3,4-didehydrocepham-4-carboxylate hemiheptahydrate [*104376-79-6*]

Ceftriaxone Sodium Hydrate contains not less than 905 μg (potency) and not more than 935 μg (potency) per mg of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$: 554.58), calculated on the anhydrous basis.

Description Ceftriaxone Sodium Hydrate is a white to yellowish white crystalline powder.
Ceftriaxone Sodium Hydrate is freely soluble in water or in dimethylsulfoxide, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Ceftriaxone Sodium Hydrate and Ceftriaxone Sodium RS (1 in 100000) as directed under Ultraviolet-visible Spectrophootmetry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Ceftriaxone Sodium Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C, and D, at around δ 3.5 ppm, at around δ 3.8 ppm, at around δ6.7 ppm, and at around δ 7.2 ppm. The ratio of the integrated intensity of each signal, A : B : C : D, is 3 : 3 : 1 : 2. When the signal at around δ 3.5 ppm overlaps with the signal of water, perform the measurement in the probe kept at about 50 °C.

(3) Ceftriaxone Sodium Hydrate responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -153 ~ -170° (50 mg calculated on the anhydrous basis, water, 2.5 mL, 20 mm)

pH The pH of a solution obtained by dissolving 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the solution is clear and light yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances 1***—Dissolve 20 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of water and acetonitrile (11 : 9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solu-

tion as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the peak area of related substance I, having the relative retention time of about 0.5 with respect to ceftriaxone from the test solution, and the peak area of related substance II, having the relative retention time of about 1.3, are not larger than the peak area of ceftriaxone from the standard solution. Use the peak areas of related substances I and II after multiplying by their relative response factors, 0.9 and 1.2, respectively.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.0 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile. To this solution add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the test solution, add a mixture of water and acetonitrile (11 : 9) to make exactly 200 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add a mixture of water and acetonitrile (11 : 9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1 % of the peak area of ceftriaxone from 10 μL of the system suitability solution.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of water and acetonitrile (11 : 9) to make 5 mL. To this solution add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile (11 : 9) (9 in 5000), and add a mixture of water and acetonitrile (11 : 9) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of ceftriaxone is not more than 1.0 %.

Time span of measurement: About 2 times as long as the retention time of ceftriaxone

(5) ***Related substance 2***—Dissolve 10 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of acetonitrile and water (23 : 11) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the peak area of each related substance eluted after the peak of ceftriaxone from the test solution is not larger than the peak area of ceftriaxone from the standard solution, and the total area of the peaks of related substances is not larger than 2.5 times the peak area from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.0 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile. To this solution add 490 mL of water, 55 mL of solution A, 5 mL of solution B, and 700 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 3 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the test solution, add a mixture of acetonitrile and water (23 : 11) to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add a mixture of acetonitrile and water (23 : 11) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1 % of the peak area of ceftriaxone from 10 μL of the system suitability solution.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of acetonitrile and water (23 : 11) to make 5 mL. To this solution add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile (11 : 9) (9 in 5000), and add a mixture of acetonitrile and water (23 : 11) to make 200 mL. When the procedure is run with 10 μL of this solu-

tion under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of ceftriaxone is not more than 1.0 %.

Time span of measurement: About 10 times as long as the retention time of ceftriaxone

Water 8.0 ~ 11.0 % (0.15 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Ceftriaxone Sodium Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of ceftriaxone, when Ceftriaxone Sodium Hydrate is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) each of Ceftriaxone Sodium Hydrate and Ceftriaxone Sodium RS, and dissolve each in a mixture of water and acetonitrile (11 : 9) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile (11 : 9) to make 200 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftriaxone to that of the internal standard.

$$\text{Amount [μg (potency)] of ceftriaxone } (C_{18}H_{18}N_8O_7S_3) = \text{Amount [μg (potency)] of Ceftriaxone Sodium RS} \times \frac{Q_T}{Q_S}$$

Internal standard—A solution of diethyl terephthalate in a mixture of water and acetonitrile (11 : 9) (9 in 5000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.0 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile. To this solution add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

System suitability

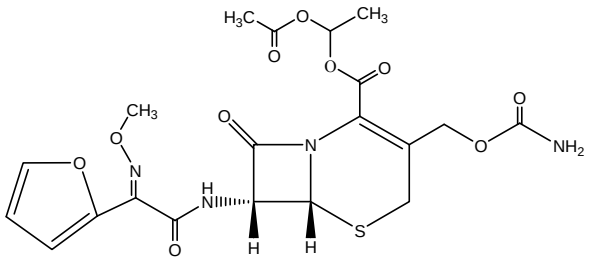
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$: 510.47

1-Acetyloxyethyl (6*R*,7*R*)-7-[(2*E*)-2-(furan-2-yl)-2-methoxyiminoacetamido]-3-carbamoyloxymethyl-3,4-didehydrocepham-4-carboxylate [*64544-07-6*]

Cefuroxime Axetil contains not less than 800 μg (potency) and not more than 850 μg (potency) per mg of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39), calculated on the anhydrous basis.

Description Cefuroxime Axetil is a white to yellowish white, non-crystalline powder.
Cefuroxime Axetil is freely soluble in dimethylsulfoxide, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectra of solutions of Cefuroxime Axetil and Cefuroxime Axetil RS (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefuroxime Axetil and Cefuroxime Axetil RS as directed in the potassium bromide disk method under Infrared Spec-

trophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefuroxime Axetil in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a pair of double signals, A, at around δ 1.5 ppm, a pair of single signals, B, at around δ 2.1 ppm, and a single signal, C, at around δ 3.9 ppm. The ratio of the integrated intensity of each signal, A : B : C, is about 1 : 1 : 1.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +41 ~ +47° (0.5 g, methanol, 50 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Put 1.0 g of Cefuroxime Axetil in a crucible, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10), and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) ***Related substances***—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make 10 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add 40 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the test solution is not larger than 1.5 times the sum area of two peaks of cefuroxime axetil obtained from the standard solution, and the total area of the peaks other than cefuroxime axetil from the test solution is not larger than 4 times the sum area of two peaks of cefuroxime axetil from the standard solution.

Internal standard solution—A solution of acetanilide in methanol (27 in 5000)

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Detection sensitivity: Pipet 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 10 mL. Confirm that the peak area of cefuroxime axetil obtained from 2 μL of this solution is equivalent to 7 to 13 % of that obtained from 2 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the sum area of the two peaks of cefuroxime axetil is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of cefuroxime axetil beginning after the solvent peak.

(4) ***Acetone***—Weigh accurately about 1 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the test solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3 %.

$$\text{Amount (\%) of acetone} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times 0.2$$

W_S: Amount (g) of acetone taken
W_T: Amount (g) of Cefuroxime Axetil taken

Internal standard solution—A solution of 1-propanol in dimethylsulfoxide (1 in 200)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A glass column about 3 mm in internal diameter and about 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1 : 1) in the ratio of 20 % (125 to 150 μm in particle diameter).

Column temperature: A constant temperature of about 90 °C

Injection port temperature: A constant temperature of about 115 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 4 minutes.

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of acetone to that of the internal standard is not more than 5.0 %.

Water Not more than 2.0 % (0.4 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (0.5 g)

Isomer Ratio Perform the test according to the Assay, and calculate the content ratio of isomer A by using the following equation (Not less than 0.48 and not more than 0.55).

$$\text{Amount of isomer A in Cefuroxime Axetil} = \frac{\text{The peak area of isomer } A}{\text{the peak area of isomer } A + \text{the peak area of isomer } B}$$

Assay Weigh accurately about 50 mg (potency) of Cefuroxime Axetil and Cefuroxime Axetil RS, dissolve each in methanol and add methanol to make exactly 50 mL. Pipet 10 mL of each solution, add exactly 5 mL of the internal standard solution and 5 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard.

$$\text{Amount [μg (potency)] of cefuroxime } (C_{16}H_{16}N_4O_8S) = \text{Amount [μg (potency)] of Cefuroxime Axetil RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acetanilide in methanol (27 in 5000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 278 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 20 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution of ammonium dihydrogen phosphate (23 in 1000) and methanol (5 : 3)

Flow rate: Adjust the flow rate so that the retention time of the peak having the smaller retention time of the two peaks of cefuroxime axetil is about 8 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard is not more than 1.0 %.

Relative retention time: The relative retention time of isomer B to the internal standard is about 2 and that of isomer A is about 2.25.

Containers and Storage ***Containers***—Tight containers. ***Storage***—Light-resistant.

Cefuroxime Axetil for Syrup

Cefuroxime Axetil for Syrup is a preparation for syrup, which is suspended before use.

Cefuroxime Axetil for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39).

Method of Preparation Prepare as directed under Syrups, with Cefuroxime Axetil.

Identification (1) Dissolve separately Cefuroxime Axetil for Syrup and Cefuroxime Axetil in methanol so that each mL contains 15.8 μg (potency), and determine the absorption spectra between 230 nm and 320 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry: the test solution and standard solution exhibit maxima at around 276 nm.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving Cefuroxime Axetil for Syrup according to the label is between 3.5 and 7.0.

Water Not more than 6.0 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Weigh accurately an amount of Cefuroxime Axetil for Syrup, equivalent to about 0.15 g (potency) according to the labeled amount, suspend according to the label, and perform the test with the suspension at 100 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of phosphate buffer solution (pH 7.0) as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add phosphate buffer solution (pH 7.0) to make exactly V' mL so that each mL contains about 20 μg (potency) of cefuroxime ($C_{16}H_{16}N_4O_8S$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 0.15 g (potency) of Cefuroxime Axetil RS, dissolve in 25 mL of methanol, add phosphate buffer solution (pH 7.0) so that each mL contains 20 μg (potency), and use this solution as the standard solution. Determine the absorbances at 278 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Cefuroxime Axetil for Syrup in 30 minutes is not less than 65 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cefuroxime } (C_{16}H_{16}N_4O_8S) = \frac{C_S}{\text{Amount (g) of Cefuroxime Axetil for Syrup taken}} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cefuroxime ($C_{16}H_{16}N_4O_8S$) in 1 g

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Cefuroxime Axetil. Weigh accurately an amount of Cefuroxime Axetil for Syrup, equivalent to about 0.15 g (potency) according to the labeled potency, add 2 mL of water, shake vigorously until dispersed, add 10.0 mL of the internal standard solution and methanol to make exactly 200 mL, and filter. Pipet 20 mL of the filtrate, add water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 15 mg (potency) of Cefuroxime Axetil RS, dissolve in 1.0 mL of the internal standard solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution.

Containers and Storage ***Containers***—Tight containers.

Cefuroxime Axetil Tablets

Cefuroxime Axetil Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39).

Method of Preparation Prepare as directed under Tablets, with Cefuroxime Axetil.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Water Not more than 6.0 % (0.2 g, volumetric titration, direct titration)

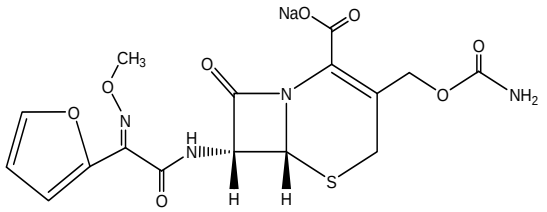
Dissolution Test Perform the test with 1 tablet of Cefuroxime Axetil Tablets at 55 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.07 mol/L hydrochloric acid solution, previously degassed using a suitable method, as the dissolution solution. Take an amount of the dissolved solution after 15 minutes from the start of the test, and immediately and carefully add the same volume of the dissolution solution, previously warmed to 37 ± 0.5 °C. Filter the dissolved solution through a membrane filter with a pore size not exceeding 0.5 μm. Pipet an amount of the filtrate, dilute with 0.07 mol/L hydrochloric acid so that each mL contains about 20 μg (potency) of cefuroxime ($C_{16}H_{16}N_4O_8S$), and use this solution as the dissolution test solution (15 minutes). Proceed with the dissolved solution after 45 minutes from the start of the test in the same manner, and use the solution thus obtained as the dissolution test solution (45 minutes). Separately, weigh accurately about 60 mg (potency) of Cefuroxime Axetil RS, dissolve in 5 mL of methanol, add 0.07 mol/L hydrochloric acid so that each mL contains 20 μg (potency), and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the test solutions and standard solution as directd under Ultraviolet-visible Spectrophotometry. The dissolution rates of Cefuroxime Axetil Tablets in 15 minutes and in 45 minutes are not less than 65 % and not less than 80 %, respectively.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Cefuroxime Axetil. Weigh accurately and powder not less than 20 Cefuroxime Axetil Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in methanol to make exactly 50 mL, pipet 10 mL of this solution, add 5 mL of the internal standard solution and 5 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cefuroxime Sodium



$C_{16}H_{15}N_4NaO_8S$: 446.37

Sodium (6*R*,7*R*)-7-[(2*E*)-2-(furan-2-yl)-2-methoxyiminoacetamido]-3-carbamoyloxymethyl-3,4-didehydrocepham-4-carboxylate [*56238-63-2*]

Cefuroxime Sodium contains not less than 875 μg (potency) per mg of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39), calculated on the anhydrous basis.

Description Cefuroxime Sodium appears as white to pale yellowish white crystals or crystalline powder.
Cefuroxime Sodium is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cefuroxime Sodium and Cefuroxime Sodium RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cefuroxime Sodium and Cefuroxime Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefuroxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate as an internal reference compound: it exhibits a single signal, A, at around δ 4.0 ppm, a quadruplet signal, B, at around δ 6.6 ppm, and double signals, C and C, at around δ 6.9 ppm and at around δ 7.7 ppm, respectively. The ratio of the integrated intensity of each signal, A : B : C : D, is 3 : 1 : 1 : 1.

(4) Cefuroxime Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +59 ~ + 66°(0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1 g of Cefuroxime Sodium in 10 mL of water is between 6.0 and 8.5.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Cefuroxime Sodium in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 450 nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.25.

(2) ***Heavy metals***—Proceed with 1.0 g of Cefuroxime Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cefuroxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 25 mg of Cefuroxime Sodium in 25 mL of water, and use this solution as the test solution. To 1 mL of the test solution add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than cefuroxime from the test solution is not larger than the peak area of cefuroxime from the standard solution, and the total area of the peaks other than cefuroxime from the test solution is not larger than 3 times the peak area of cefuroxime from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of cefuroxime obtained from 20 μL of this solution is equivalent to 7 to 13 % of the peak area of cefuroxime from the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefuroxime is not more than 2.0 %.

Time span of measurement: About 4 times as long as the retention time of cefuroxime beginning after the solvent peak.

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cefuroxime Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet

5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylan iline taken} \times \text{content (\%) of dimethylan iline}}{\text{Amount (mg) of Cefuroxime Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with silanized diatomaceous earth for gas chromatography coated with 50 % phenyl-50 5 methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 4.0 % (0.4 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Cefuroxime Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefuroxime, when Cefuroxime Sodium is used in a sterile preparation.

Assay Weigh accurately about 25 mg (potency) each of Cefuroxime Sodium and Cefuroxime Sodium RS, dissolve in water to make exactly 25 mL, and use these solutions as the test solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefuroxime.

$$\text{Amount [μg (potency)] of cefuroxime } (C_{16}H_{16}N_4O_8S) = \text{Amount [μg (potency)] of Cefuroxime Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 273 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 125 mm in length, packed with hexasilyl silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.68 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 3.4 with acetic acid (100), and add water to make 1000 mL. To 990 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefuroxime is about 8 minutes.

System suitability

System performance: Allow the test solution to stand at 60 °C for 10 minutes, and cool. When the procedure is run quickly with 20 μL of this solution under the above operating conditions, the resolution between the peak of cefuroxime and the peak with the relative retention time of about 0.7 with respect to the peak of cefuroxime is not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefuroxime is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cefuroxime Sodium for Injection

Cefuroxime Sodium for Injection is a preparation for injection, which is dissolved before use.
Cefuroxime Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39).

Method of Preparation Prepare as directed under Injections, with Cefuroxime Sodium.

Description Cefuroxime Sodium for Injection appears as white to pale yellowish white powder.

Identification Perform the test as directed in the Identification (1) and (2) under Cefuroxime Sodium.

pH The pH of a solution obtained by dissolving an amount of Cefuroxime Sodium for Injection, equivalent to 1.0 g (potency) of cefuroxime, in 10 mL of water is between 6.0 and 8.5.

Water Not more than 3.5 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of cefuroxime.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately about 50 mg (potency) of Cefuroxime Sodium for Injection, according to the labeled potency, dissolve in water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Cefuroxime Sodium RS, dissolve in water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefuroxime sodium in the test solution and standard solution.

$$\text{Amount [μg (potency)] of cefuroxime } (C_{16}H_{16}N_4O_8S) = \text{Amount [μg (potency)] of Cefuroxime Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

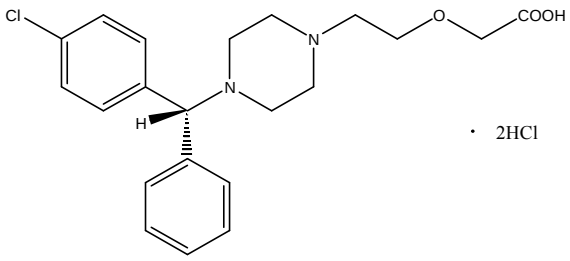
Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with hexylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of pH 3.4 acetate buffer solution (to 50 mL of 0.1 mol/L sodium acetate add 0.1 mol/L acetic acid to make 1000 mL) and acetonitrile (10 : 1)

Flow rate: 2.0 mL/minute

Containers and Storage ***Containers***—Hermetic containers.

Cetirizine Dihydrochloride



and enantiomer

$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$: 461.81

2-[2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy]acetic acid dihydrochloride [*83881-52-1*]

Cetirizine Dihydrochloride contains not less than 99.0 % and not more than 100.5 % of cetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$), calculated on an anhydrous basis.

Description Cetirizine Dihydrochloride is a white powder.

Cetirizine Dihydrochloride is freely soluble in water, and practically insoluble in acetone or in methylene chloride.

Identification (1) Dissolve 20.0 mg of Cetirizine Dihydrochloride in 0.1 mol/L hydrochloric acid to make 100 mL. Dilute 10.0 mL of this solution with 0.1 mol/L hydrochloric acid to 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry. Absorption maximum occurs at 231 nm, and the specific absorbance at this wavelength ranges from 359 to 381.

(2) Determine the infrared spectra of Cetirizine Dihydrochloride and Cetirizine Dihydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 10 mg of Cetirizine Dihydrochloride in water to make 5 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Cetirizine Dihydrochloride RS in water to make 5 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of chlorphenamine maleate RS in water to make 5 mL. To 1 mL of the solution, add 1 mL of the standard solution (1). Use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of methylene chloride, methanol, and ammonia solution (28) (90 : 10 : 1) to a distance of about 15 cm, and dry the plate in cool air. Examine in ultraviolet light at 254 nm, and compare the principal spot from the test solution with that from the standard

solution (1). Their R_f values are the same. The test is valid when 2 clearly separated spots are observed with the standard solution (2).

(4) The solution of Cetirizine Dihydrochloride in water (1 in 100) responds to the Qualitative Tests for chloride (2nd method).

pH The pH of a solution obtained by dissolving 1.0 g of Cetirizine Dihydrochloride in 20 mL of water is between 1.2 and 1.8.

Purity (1) ***Clarity and color of solution*—**When 2.0 g of Cetirizine Dihydrochloride is dissolved in 20 mL of water, the resultant solution is clear.

(2) ***Heavy metals* —**Proceed the test with 2.0 g of Cetirizine Dihydrochloride according to Method 1. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances*—**Dissolve 20.0 mg of Cetirizine Dihydrochloride in the mobile phase to make 100 mL, and use this solution as the test solution. Separately, dissolve 5.0 mg of Cetirizine Dihydrochloride RS and 5.0 mg of cetirizine related substance I RS [(*RS*)-1-[(4-chlorophenyl)phenylmethyl] piperazine} in the mobile phase to make 25 mL. Dilute 1.0 mL of the solution to 100 mL with the mobile phase, and use this solution as the standard solution (1). Dilute 2.0 mL of the test solution to 50 mL with the mobile phase. Dilute 5.0 mL of this solution to 100 mL with the mobile phase, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution, the standard solutions (1), and (2) as directed under Liquid Chromatography according to the following conditions. Determine the area of each peak by the automatic integration method. The peak area of any related substance, excluding the principal peak area corresponding to cetirizine, from the test solution is not more that the area of the principal peak from the standard solution (2) (0.2 %); and the sum of the peak areas of all related substances from the test solution is not more than 1.5 times the area of the principal peak from the standard solution (2) (0.3 %). Disregard any peak areas that are not more than 0.1 times the principal peak area from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile, water, and dilute sulfuric acid (93 : 6.6 : 0.4).

Flow rate: 1.0 mL/min.

System suitability

System performance: Adjust the sensitivity of the system so that the peak heights of cetirizine and the related substance I are about 50 % of the full scale of the recorder. When the procedure is run with 20 μL of the standard solution (1) under the above operation conditions, the resolution between the peaks of cetirizine and the related substance I is not less 3, and the symmetry factor is not more than 2.0.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, constant weight).

Residue on Ignitio Not more than 0.2 % (1 g).

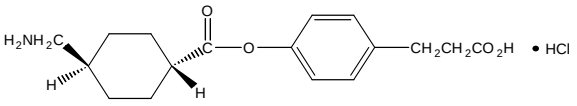
Assay Dissolve 0.100 g of Cetirizine Dihydrochloride in 70 mL of a mixture of water and acetone (30 : 70), titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.394 mg of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cetraxate Hydrochloride



$C_{17}H_{23}NO_4 \cdot HCl$: 341.83

3-[4-[4-(Aminomethyl)cyclohexanecarbonyl] oxyphenyl]propanoic acid hydrochloride [*27724-96-5*]

Cetraxate Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of cetraxate hydrochloride ($C_{17}H_{23}NO_4 \cdot HCl$).

Description Cetraxate Hydrochloride appears as white crystals or crystalline powder.

Cetraxate Hydrochloride is soluble in methanol, sparingly soluble in water or in ethanol (95) and practically insoluble in ether.

Melting point —About 236 ºC (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Cetraxate Hydrochloride and Cetraxate Hydrochloride RS in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.5 g of Cetraxate Hydrochloride in 5 mL of a mixture of water and 2-propanol (1 : 1) by warming, cool to below 25 °C. Filter, dry the formed crystals in vacuum for 4 hours, and further dry at 105 °C for 1 hour. Determine the infrared spectra of the

dried matter and Cetraxate Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Cetraxate Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cetraxate Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Cetraxate Hydrochloride according to Method 3 and perform the test with a solution of magnesium nitrate in ethanol (95) (1 in 5) (not more than 2 ppm).

(3) ***Cis isomer***—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of water and use this solution as the test solution. To exactly 5 mL of the test solution, add water to make exactly 100 mL. To exactly 2 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: the area of the peak which has a retention time 1.3 to 1.6 times that of cetraxate from the test solution is not larger than the peak area of cetraxate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column, about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15 : 10 : 4) to 6.0 with acetic acid.

Flow rate: Adjust the flow rate so that the retention time of cetraxate is about 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cetraxate obtained from 10 μL of the standard solution is not less than 20 mm.

System performance: Dissolve 20 mg of Cetraxate Hydrochloride and 10 mg of phenol in 100 mL of water. To 2 mL of this solution, add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetraxate and phenol are eluted in this order with the resolution between these peaks being not less than 5.

(4) ***3-(p-Hydroxyphenyl)propionic acid***—To about 0.10 g of Cetraxate Hydrochloride, add exactly 2 mL of the internal standard solution and methanol to make 10 mL and use this solution as the test solution. Separately, dissolve 25 mg of 3-(p-hydroxyphenyl)propionic acid in methanol to make exactly 100 mL. To exactly 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratio, Q_T and Q_S, of the peak area of 3-(p-hydroxyphenyl) propionic acid to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of Caffeine in methanol (1 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column, about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15 : 5 : 2) to 5.5 with acetic acid.

Flow rate: Adjust the flow rate so that the retention time of 3-(p-hydroxyphenyl)propionic acid is about 7 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 3-(p-hydroxy phenyl) propionic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 3-(p-hydroxyphenyl) propionic acid obtained from 10 μL of the standard solution is not less than 30 mm.

(5) ***Related substances***—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and acetic acid (100) (20 : 4 : 3) to a distance of about 10 cm and air-dry the plate. Spray evenly ninhydrin TS on the plate and heat at 90 ºC for 10 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, 105 ºC, 3 hours).

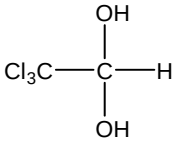
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.5 g of Cetraxate Hydrochloride, previously dried, dissolve in 100 mL of water and adjust the pH of this solution to between 7.0 and 7.5 with dilute sodium hydroxide TS. To this solution, add 10 mL of formaldehyde, stir for about 5 minutes and titrate with 0.1 mol/L sodium hydroxide VS for over about 20 minutes (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 34.183 mg of $C_{17}H_{23}NO_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Chloral Hydrate



$C_2H_3Cl_3O_2$: 165.40

2,2,2-Trichloroethane-1,1-diol [*25655-41-8*]

Chloral Hydrate contains not less than 99.5 % and not more than 101.0 % of chloral hydrate ($C_2H_3Cl_3O_2$).

Description Chloral Hydrate appears as colorless crystals, has a pungent odor and an acrid, slightly bitter taste.
Chloral Hydrate is very soluble in water and freely soluble in ethanol (95) or in ether.
Chloral Hydrate slowly volatilizes in air.

Identification (1) Dissolve 0.2 g of Chloral Hydrate in 2 mL of water and add 2 mL of sodium hydroxide TS: the turbidity is produced and it separates into two clear layers by warming.

(2) Heat 0.2 g of Chloral Hydrate with 3 drops of aniline and 3 drops of sodium hydroxide TS: the disagreeable odor of phenylisocyanide (poisonous) is perceptible.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Chloral Hydrate in 2 mL of water: the solution is clear and colorless.

(2) ***Acid***—Dissolve 0.20 g of Chloral Hydrate in 2 mL of water and add 1 drop of methyl orange TS: a yellow color is observed.

(3) ***Chloride***—Perform the test with 1.0 g of Chloral Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(4) ***Chloral alcoholate***—Warm 1.0 g of Chloral Hydrate with 10 mL of sodium hydroxide TS, filter the clear supernatant liquid, add iodine TS to the filtrate until a yellow color is observed and allow the solution to stand for 1 hour: no yellow precipitate is produced.

(5) ***Benzene***—Warm the solution obtained in (1) with 3 mL of water: no odor of benzene is perceptible.

(6) ***Heavy metals***—Dissolve 3.0 g of Chloral Hydrate in freshly boiled and cooled water to make 30 mL. Pipet 10.0 mL of this solution, add water to make 20 mL, and use this solution as the test solution. Separately, proceed with 6.0 mL of standard lead solution in the same manner as the test solution. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the control solution. Separately, to 10 mL of water add 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

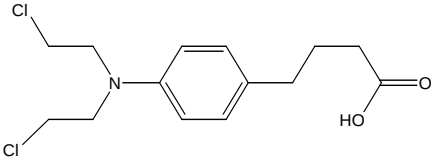
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 4 g of Chloral Hydrate in a glass-stoppered flask, add 10 mL of water and 40 mL of 1 mol/L sodium hydroxide VS and allow the mixture to stand for exactly 2 minutes. Titrate the excess sodium hydroxide immediately with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 165.40 mg of $C_2H_3Cl_3O_2$.

Containers and Storage ***Containers***—Tight containers.

Chlorambucil



$C_{14}H_{19}Cl_2NO_2$: 304.21

4-[4-[*bis*(2-Chloroethyl)amino]phenyl]butanoic acid [*305-03-3*]

Chlorambucil contains not less than 98.0 % and not

more than 101.0 % of chlorambucil ($C_{14}H_{19}Cl_2NO_2$), calculated on the anhydrous basis.

Description Chlorambucil is a milk-white granule-shaped powder.
Chlorambucil is practically insoluble in water and soluble in acetone.
Chlorambucil dissolves in dilute sodium hydroxide TS.

Identification (1) Dissolve 50 mg of Chlorambucil in 5 mL of acetone and dilute with water to make 10 mL. Add 1 drop of 1 mol/L sulfuric acid and, then, add 4 drops of silver nitrate TS: no opalescence is observed immediately. Warm this solution on a steam-bath: opalescence develops.

(2) Determine the infrared spectra of solutions of Chlorambucil and Chlorambucil RS in carbon disulfide (1 to 125) as directed under Infrared Spectrophotometry, using solid cell with 1 mm of thickness: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 65 ~ 69 ºC.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.2 g of Chlorambucil, dissolve in 10 mL of acetone, add 10 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 30.421 mg of $C_{14}H_{19}Cl_2NO_2$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Chlorambucil Tablets

Chlorambucil Tablets contain not less than 85.0 % and not more than 110.0 % of the labeled amount of the chlorambucil ($C_{14}H_{19}Cl_2NO_2$: 304.22).

Preparation Prepare as directed under Tablets, with Chlorambucil.

Identification Weigh a portion of powered Chlorambucil Tablets, equivalent to 16 mg of Chlorambucil, add 20 mL of carbon disulfide and shake to mix. Filter and evaporate the filtrate to dryness. Dissolve the residue in 2 mL of carbon disulfide. Proceed the test with the solution as directed in the Identification (1) under Chlorambucil.

Disintegration Test Place 1 tablet in each of six tubes of baskets. If the tablet has a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Operate the apparatus, using the first solution maintained at 37 ± 2 ºC as the immersion fluid. After 30 minutes of operation in the first solution, lift the basket from the fluid, and observe the tablets. If the tablets have not disintegrated completely, substitute the second solution maintained at 37 ± 2 ºC as the immersion fluid, and continue the test for total period time, including previous exposure to water and the first solution, equal to 45 minutes. If one or two tablets do not disintegrate, proceed the test with additional 12 tablets and determine: tablets which do not disintegrate are not more than 2 out of total 18 tablets.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Chlorambucil Tablets. Weigh accurately a portion of the powder, equivalent to about 2 mg of chlorambucil ($C_{14}H_{19}Cl_2NO_2$), add 50 mL of ethanol (95), shake cautiously, add 5.0 mL of 0.1 mol/L hydrochloric acid and 2.0 mL of internal standard solution, shake by mechanical means for 5 minutes and dilute with ethanol (95) to make exactly 100 mL. Filter and use this filtrate as the test solution. Separately, weigh accurately about 20 mg of Chlorambucil RS, previously dried in a desiccators (silica gel) for 24 hours and dissolve in ethanol (95) to make exactly 20 mL. To 2.0 mL of this solution, add 50 mL of ethanol (95), 5.0 mL of 0.1 mol/L hydrochloric acid and 2.0 mL of internal standard solution with cautious shaking and dilute with ethanol (95) to make exactly 100 mL. Use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the relative peak areas compared to internal standard, Q_T and Q_S, of Chlorambucil of the test solution and the standard solution, respectively.

$$\text{Amounts (mg) of chlorambucil } (C_{14}H_{19}Cl_2NO_2) = \text{Amount (mg) of Chlorambucil RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of propyl *p*-oxybenzoate in ethanol (1 in 2500).

Operating Conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 2 mm in internal diameter and about 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: Mix 500 mL of ethanol (95) and 1.0 mL of acetic acid (100) and add water to make 1000 mL.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between peaks of

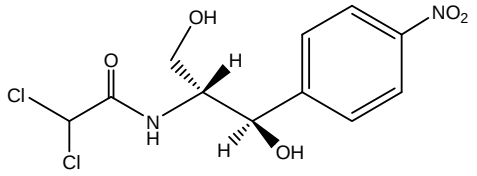
chlorambucil and internal substance is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak area of chlorambucil is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant (in case of uncoated tablets).

Chloramphenicol



$C_{11}H_{12}Cl_2N_2O_5$: 323.13

2,2-Dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide [*56-75-7*]

Chloramphenicol contains not less than 980 μg (potency) and not more than 1020 μg (potency) per mg of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$), calculated on the dried basis.

Description Chloramphenicol appears as whtie to yellowish white crystals or crystalline powder, is odorless, and has a bitter taste.
Chloramphenicol is freely soluble in methanol or in ethanol (99.5), and slightly soluble in water.

Identification (1) Determine the absorption spectra of the test solution and standard solution of Chloramphenicol and Chloramphenicol RS, respectively, obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chloramphenicol and Chloramphenicol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +18.5 ~ +21.5° (1.25 g, ethanol (99.5), 25 mL, 100 mm).

Melting Point 150 ~ 155 °C.

pH The pH of a saturated solution of Chloramphenicol in water is between 4.5 and 7.5.

Absorbance $E_{1cm}^{1\%}$ (278 nm): 289 ~ 307 (20 mg, water, 1000 mL).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chloramphenicol according to Method 2 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 25 ppm).

(2) ***Arsenic***—Proceed with 2.0 g of Chloramphenicol according to Method 4, and perform the test (not more than 1 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Chloramphenicol in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of this solution, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, and acetic acid (100) (79 : 14 : 7) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point obtained from the test solution are not more intense than the spot from the standard solution (1), and the total amount of these spots is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Sterility Test It meets the requirement, when Chloramphenicol is used in a sterile preparation.

Bacterial Endotoxins Less than 0.2 EU/mg (potency) of chloramphenicol, when Chloramphenicol is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Chloramphenicol and Chloramphenicol RS, dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of chloramphenicol.

$$\text{Amount [μg (potency)] of chloramphenicol } (C_{11}H_{12}Cl_2N_2O_5) = \text{Amount [μg (potency)] of Chloramphenicol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 250 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: A mixture of water, methanol and acetic acid (31) (550:450:1)

Flow rate: 1.5 mL per minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of chloramphenicol are not less than 1800 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Chloramphenicol Capsules

Chloramphenicol Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Method of Preparation Prepare as directed under Capsules, with Chloramphenicol.

Identification (1) Dissolve an amount of the contents of Chloramphenicol Capsules, equivalent to 10 mg of chloramphenicol, in 1 mL of 50 % ethanol (95), add 3 mL of a solution of calcium chloride (1 in 100) and 50 mg of zinc powder, and heat in a water bath for 10 minutes. Pour the clear supernatant liquid into a test tube, add 0.1 g of anhydrous sodium acetate and 2 drops of benzoyl chloride, shake for 1 minute, and add 10 drops of iron (III) chloride (if the solution is not clear, add dilute hydrochloric acid): a purple to red-purple color develops. When the above test is performed without addition of zinc powder, the solution is colorless.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Dissolution Test Perform the test with 1 capsule of Chloramphenicol Capsules at 100 revolutions per minute according to Method 1, using 900 mL of 0.01 mol/L hydrochloric acid TS as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Chloramphenicol RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Chloramphenicol Capsules in 30 minutes is not less than 85 % (Q).

Dissolution rate (%) with respect to the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Chloramphenicol. Weigh accurately the mass of the contents of not less than 20 Chloramphenicol Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Chloramphenicol Ophthalmic Solution

Chloramphenicol Ophthalmic Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Method of Preparation Prepare as directed under Ophthalmic Solutions, with Chloramphenicol.

Identification (1) Weigh an amount of Chloramphenicol Ophthalmic Solution, equivalent to 10 mg of chloramphenicol, dissolve in 1 mL of 50 % ethanol (95), add 3 mL of a solution of calcium chloride (1 in 100) and 50 mg of zinc powder, and heat in a water bath for 10 minutes. Pour the clear supernatant liquid into a test tube, add 0.1 g of anhydrous sodium acetate and 2 drops of benzoyl chloride, shake for 1 minute, and add 10 drops of iron (III) chloride TS (if the solution is not clear, add dilute hydrochloric acid): a purple to red-purple color develops. When the above test is

performed without addition of zinc powder, the solution is colorless.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH 3.0 ~ 6.0. If a buffer is used, the pH is between 7.0 and 7.5.

Sterility Test It meets the requirement.

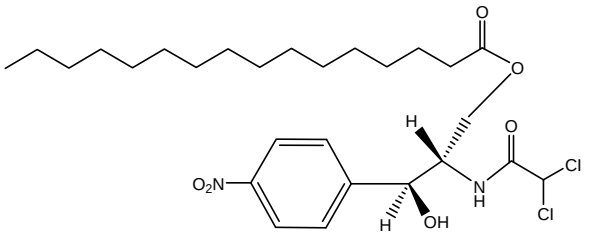
Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Ophthalmic Solutions It meets the requirement.

Assay Proceed as directed in the Assay under Chloramphenicol. Weigh accurately an amount of Chloramphenicol Ophthalmic Solution, equivalent to about 40 mg (potency) according to the labeled potency, add methanol to make exactly 100 mL, pipet 5 mL of this solution, add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the test solution. Separately, weigh accurately about 40 mg (potency) of Chloramphenicol RS, dissolve in 15 mL of water and 75 mL of methanol, add methanol to make exactly 100 mL, pipet 5 mL of this solution, add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Chloramphenicol Palmitate



$C_{27}H_{42}Cl_2N_2O_6$: 561.54

[(2*R*,3*R*)-2-[(2,2-Dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl]hexadecanoate [*530-43-8*]

Chloramphenicol Palmitate contains not less than 558 μg (potency) and not more than 587 μg (potency) per mg of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13), calculated on the dried basis.

Description Chloramphenicol Palmitate appears as white to grayish white crystalline powder.

Chloramphenicol Palmitate is freely soluble in acetone, sparingly soluble in methanol or in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS in ethanol (99.5) (1 in 33000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve separately 5 mg each of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS in 1 mL of acetone, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and cyclohexane (1 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the test solution shows the same R_f value as the spot from the standard solution.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +21 ~ +25° (1 g calculated on the dried basis, ethanol (99.5), 20 mL, 100 mm).

Melting Point 91 ~ 96 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately 50 mg of Chloramphenicol Palmitate, dissolve in methanol to make 50 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. The test should be performed within 30 minutes after the test solution and standard solution are prepared. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than chloramphenicol palmitate from the test solution is not larger than 3.5 times the peak area of chloramphenicol palmitate from the standard solution. Use the peak areas of chloramphenicol and chloramphenicol dipalmitate, having the relative retention times of about 0.5 and about 5.0 with respect to chloramphenicol palmitate, after multiplying by their relative response factors, 0.5 and 1.4, respectively.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20 °C

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of chloramphenicol palmitate

System suitability

Test for required detectability: Dissolve 50 mg of Chloramphenicol Palmitate in methanol to make 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 5 mL of the system suitability solution, and add methanol to make exactly 50 mL. Confirm that the peak area of chloramphenicol palmitate obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 5000.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of chloramphenicol palmitate is not more than 1.0 %.

(4) ***Free chloramphenicol***—Weigh accurately about 1.0 g of Chloramphenicol Palmitate, and dissolve in xylene by heating to make exactly 80 mL. After cooling, extract with three 15 mL volumes of water and discard the xylene. To the extract add water to make exactly 50 mL. To this solution add exactly 10 mL of toluene, mix, and extract the water layer. Determine the absorbance, A, at 278 nm of this solution as directed under Ultraviolet-visible Spectrophotometry: not more than 0.268. Perform a blank determination and make any necessary correction (not more than 450 ppm).

$$\text{Free chloramphenicol (ppm)} = A \times \frac{10000}{5.96}$$

Loss on Drying Not more than 1.0 % (1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Assay Weigh accurately about 37 mg (potency) each of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS, dissolve each in 40 mL of methanol and 1 mL of acetic acid (100), and add methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of chloramphenicol palmitate in each solution.

$$\text{Amount [μg (potency)] of chloramphenicol } (C_{11}H_{12}Cl_2N_2O_5) = \text{Amount [μg (potency)] Chloramphenicol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of methanol, water, and acetic acid (100) (172 : 27 : 1)

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 7 minutes.

System suitability

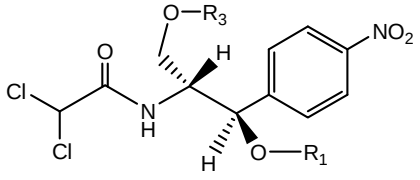
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 2400.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chloramphenicol Sodium Succinate



$C_{15}H_{15}Cl_2N_2NaO_8$: 445.18

Sodium 4-[(2*R*,3*R*)-2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propoxy]-4-oxobutanoate [*982-57-0*]

Chloramphenicol Sodium Succinate contains not less than 711 μg (potency) of per mg of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13), calculated on the anhydrous basis.

Description Chloramphenicol Sodium Succinate appears as white to yellowish white crystals or crystalline powder.
Chloramphenicol Sodium Succinate is very soluble in water, and freely soluble in methanol or in ethanol (99.5).
Chloramphenicol Sodium Succinate is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Chloramphenicol Sodium Succinate and Chloramphenicol Sodium Succinate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chloramphenicol Sodium Succinate and Chloramphenicol Sodium Succinate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Chloramphenicol Sodium Succinate responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +5 ~ +8° (1.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.4 g of Chloramphenicol Sodium Succinate in 5 mL of water is between 6.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear and colorless to yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 1, and perform the test (not more than 2 ppm).

(4) ***Free chloramphenicol***—Weigh accurately about 33 mg of Chloramphenicol Sodium Succinate, dissolve in the mobile phase to make exactly 50 mL, filter through a filter with a pore size not exceeding 0.5 μm, and use the filtrate as the test solution. Weigh accurately about 0.6 mg of Chloramphenicol RS, dissolve in the mobile phase to make exactly 100 mL, filter through a filter with a pore size not exceeding 0.5 μm, and use the filtrate as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of each solution by the automatic integration method, and determine the peak areas, A_T and A_S, of chloramphenicol in each solution (not more than 2.0 %).

$$\text{Amount (\%) of free chloramphenicol } (C_{11}H_{12}Cl_2N_2O_5) = 5000 \times \frac{C}{W \times Q} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of chloramphenicol in the standard solution

Q: Amount (μg) of chloramphenicol in each mg of chloramphenicol sodium succinate in the test solution

W: Amount (mg) of Chloramphenicol Sodium Succinate taken

A_T: Peak area of chloramphenicol from the test solution

A_S: Peak area of chloramphenicol from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 0.05 mol/L ammonium dihydrogen phosphate TS (pH adjusted to 2.5 ± 0.1 with 10 % phosphoric acid) and methanol (60 : 40).

Flow rate: 1 mL/minute

System suitability

Column performance: When the procedure is run with the test solution under the above operating conditions, the resolution between the two principal peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not less than 2.0, and the number of theoretical plates and symmetry factor are not less than 1750 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of the two principal peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not more than 2.0 %.

Sterility Test It meets the requirement, when Chloramphenicol Sodium Succinate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.2 EU/mg of chloramphenicol, when Chloramphenicol Sodium Succinate is used in a sterile preparation.

Water Not more than 2.0 % (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately about 20 mg (potency) of Chloramphenicol Sodium Succinate, dissolve in water to make exactly 1000 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg

(potency) of Chloramphenicol Succinate RS, and suspend in exactly 50 mL of water. Add slowly 7 mL of 0.01 mol/L sodium hydroxide TS while stirring, and adjust the pH to 7.0. To this solution add water to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 276 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount [μg (potency)] of chloramphenicol } (C_{11}H_{12}Cl_2N_2O_5) = \text{Amount [μg (potency)] Chloramphenicol Sodium Succinate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Hermetic containers.

Chloramphenicol Sodium Succinate for Injection

Chloramphenicol Sodium Succinate for Injection is a preparation for injection, which is dissolved before use. Chloramphenicol Sodium Succinate for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Method of Preparation Prepare as directed under Injections, with Chloramphenicol Sodium Succinate.

Description Chloramphenicol Sodium Succinate for Injection appears as white to yellowish white powder.

Identification (1) To an amount of Chloramphenicol Sodium Succinate for Injection, equivalent to about 50 mg of chloramphenicol sodium succinate, add 5 mL of pyridine and 5 mL of 1 mol/L sodium hydroxide TS, shake, and heat in a water bath for several minutes: the pyridine layer develops a deep red color.

(2) To an amount of Chloramphenicol Sodium Succinate for Injection, equivalent to about 0.1 g of chloramphenicol sodium succinate, add 0.2 g of resorcinol and 0.2 mL of sulfuric acid, heat until the color of the solution becomes deep red, and add this solution slowly to an excess of water: an orange color with a green fluorescence develops.

(3) Determine the absorption spectra of solutions of Chloramphenicol Sodium Succinate for Injection and Chloramphenicol Sodium Succinate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit a maximum between 271 and 281 nm.

pH Dissolve an amount of Chloramphenicol Sodium Succinate for Injection, equivalent to 2.5 g (potency) of chloramphenicol, in 10 mL of water: the pH of the solution is between 6.4 and 7.0.

Purity ***Free choramphenicol***—Weigh accurately an amount of Chloramphenicol Sodium Succinate for Injection, equivalent to about 1 g, and dissolve in the mobile phase to make exactly 10 mL. Pipet 1 mL of this solution, dissolve in the mobile phase to make exactly 200 mL, filter through a filter with a pore size of 0.5 μm, and use the filtrate as the test solution. Weigh accurately about 0.6 mg of Chloramphenicol RS, dissolve in the mobile phase to make exactly 100 mL, filter through a filter with a pore size of 0.5 μm, and use the filtrate as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of each solution by the automatic integration method, and determine the peak areas, A_T and A_S, of chloramphenicol in each solution (not more than 2.0 %).

$$\text{Amount (\%) of free chloramphenicol } (C_{11}H_{12}Cl_2N_2O_5) = 0.1 \times \frac{C}{D} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of chloramphenicol in the standard solution

D: Concentration (μg/mL) of chloramphenicol in the test solution

A_T: Peak area of chloramphenicol obtained from the test solution

*A*s: Peak area of chloramphenicol obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 0.05 mol/L ammonium dihydrogen phosphate TS (pH adjusted to 2.5 ± 0.1 with 10 % phosphoric acid) and methanol (60 : 40).

Flow rate: 1 mL/minute

System suitability

Column performance: When the procedure is run with the test solution under the above operating conditions, the resolution between the two principal peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not less than 2.0, and the number of theoretical plates and symmetry factor are not less than 1750 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of the two principal peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not more than 2.0 %.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.2 EU/mg (potency) of chloramphenicol.

Foreign Insoluble Matter Test It meets the requirement.

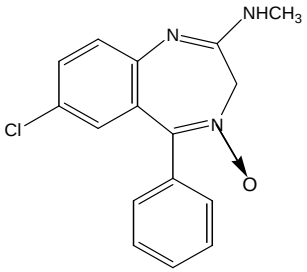
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Chloramphenicol Sodium Succinate. Weigh accurately an amount of Chloramphenicol Sodium Succinate for Injection, equivalent to about 20 mg (potency) according to the labeled potency, dissolve in water to make exactly 1000 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Chlordiazepoxide



$C_{16}H_{14}ClN_3O$: 299.76

7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide [*58-25-3*]

Chlordiazepoxide, when dried, contains not less than 98.5 % and not more than 101.0 % of chlordiazepoxide ($C_{16}H_{14}ClN_3O$).

Description Chlordiazepoxide appears as white to pale yellow crystals or crystalline powder.
Chlordiazepoxide is freely soluble in acetic acid (100), sparingly soluble in ethanol (95), very lightly soluble in ether and practically insoluble in water.
Chlorodiazepoxide dissolves in dilute hydrochloric acid.
Chlorodiazepoxide is gradually affected by light.

Melting point— About 240 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Chlordiazepoxide and Chlordiazepoxide RS in 0.1 mol/L hydrochloric acid TS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chlordiazepoxide and Chlordiazepoxide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Proceed with Chlordiazepoxide as directed under the Flame Coloration Test (2) and perform the test: a green color develops.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chlordiazepoxide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substance***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 0.20 g of Chlordiazepoxide in exactly 10 mL of a mixture of methanol and ammonia TS (97 : 3) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of methanol and ammonia TS (97 : 3) to make exactly 200 mL and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-amino-5-chlorobenzophenone RS in methanol to make exactly 200 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 25 μL of the test solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (99.5) (19 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (1). Spray evenly a solution of sodium nitrite in 1 mol/L hydrochloric acid TS (1 in 100) on the plate, allow to stand for 1 minute and spray evenly *N*-(1-naphthyl)-*N'*-diethyl-ethylene-diamine oxalateacetone TS on the plate: the spots from the test solution are not more intense than the spots from the standard solution (2).

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.6 g of Chlordiazepoxide, previously dried and dissolve in 50 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS until the color of the clear supernatant liquid changes from purple through blue-purple to blue (indicator: 3 drops of methylrosaniline chloride

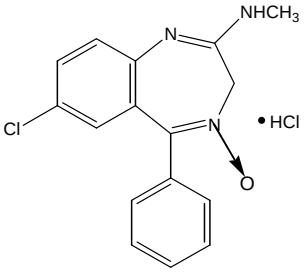
TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.976 mg of $C_{16}H_{14}ClN_3O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlordiazepoxide Hydrochloride



$C_{16}H_{14}ClN_3O \cdot HCl$: 336.22

7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide monohydrochloride [*438-41-5*]

Chlordiazepoxide Hydrochloride contains not less than 98.0 % and not more than 102.0 % of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$), calculated on the dried basis.

Description Chlordiazepoxide Hydrochloride is a white crystalline powder and is odorless.
Chlordiazepoxide Hydrochloride is soluble in water or in ethanol (95) and sparingly soluble in hexane.
Chlordiazepoxide Hydrochloride is affected by light.

Identification (1) To about 20 mg of Chlordiazepoxide Hydrochloride, add 5 mL of hydrochloric acid and 10 mL of water and heat to boiling to hydrolyze. To the cooled solution, add 2 mL of sodium nitrite solution (1 in 1000), 1 mL of ammonium sulfamate solution (1 in 200) and 1 mL of *N*-1-naphthylethylenediamine dihydrochloride solution (1 in 1000): a red-purple color is observed.

(2) The relative retention time of the major peak in the chromatogram of the test solution corresponds to that of the Standard solution obtained as directed in the Assay.

(3) Determine the infrared spectra of Chlordiazepoxide Hydrochloride and Chlordiazepoxide Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 212 ~ 218 ºC (with decomposition).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chlordiazepoxide Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Transfer about 50 mg of Chlordiazepoxide Hydrochloride to a volumetric flask and dissolve in 2.5 mL of acetone by shaking. Allow to stand until precipitate and use the clear supernatant liquid as the test solution. Separately, weigh a portion of 7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one-4-oxide RS, dissolve in acetone to make the concentration of 100 μg per mL and use this solution as the standard solution (1). Weigh a portion of 2-Amino-5-chlorobenzophenone RS, dissolve in acetone to make the solution containing 10 μg per mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 50 μL of the test solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate in a developing chamber, previously not equilibrated with the developer, with ethyl acetate until the solvent front has moved about three-fourths of the length of the plate, air-dry the plate. Spray evenly 1 mol/L sulfuric acid in the plate, dry the plate at 105 ºC for 15 minutes and then spray sodium nitrite (1 in 1000), ammonium sulfamate (1 in 200) and *N*-(1-naphthyl)ethylenediamine hydrochloric acid (1 in 1000) sequentially. The spots, equivalent to those from the standard solutions, from the test solution are not more intense than the spots from the standard solutions (1) and (2).

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Perform the test under the conditions protected from light. Transfer about 0.1 g of Chlordiazepoxide Hydrochloride, accurately weighed, to a volumetric flask, dissolve in mobile phase by sonicating for minutes, dilute with mobile phase to make 50 mL and mix. Transfer 10 mL of the resulting solution, dilute with mobile phase to make 100 mL, mix and use this solution as the test solution. Separately, weigh a portion of Chlordiazepoxide Hydrochloride RS and accurately dissolve in mobile phase to make the concentration of 1 mg per mL. Pipet 10 mL of this solution, add mobile phase to make exactly 100 mL and use this solution as a standard solution. Perform the test with 5 μL each of the test solution and the standard solution and calculate the peak areas, A_T and A_S, the test solution and the standard solution respectively.

Amount (mg) of Chlordiazepoxide Hydrochloride

$$(C_{16}H_{14}ClN_3O\cdot HCl) = 0.5 \times C \times \frac{A_T}{A_S}$$

C: Concentration of Chlordiazepoxide Hydrochloride RS solution (μg/mL).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture of water and methanol (60 : 40).

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 3600 and not more than 2.0, respectively.

System repeatability: When the test is repeated 5 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlordiazepoxide hydrochloride is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlordiazepoxide Hydrochloride Capsules

Chlordiazepoxide Hydrochloride Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$: 336.22).

Method of Preparation Prepare as directed under Capsules, with Chlordiazepoxide Hydrochloride.

Identification (1) The solution used for measurement of absorbance in the Assay exhibits maxima at 245 ± 2 nm and 311 ± 2 nm and the ratio, A_{245}/A_{311}, is between 2.90 and 3.45.

(2) Proceed with a suitable amount of the contents of Chlordiazepoxide Hydrochloride Capsules as directed it the Identification (1) under Chlordiazepoxide Hydrochloride.

Purity ***Related substances***—Accurately weigh a portion of Chlordiazepoxide Hydrochloride Capsules, equivalent to about 25 mg of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$) and perform the test as directed in Purity (2) under Chlordiazepoxide Hydrochloride. Use 15 μL of 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzo-diazepin-2-one-4-oxide in acetone (1 in 1000) and 10 μL of 2-amino-5-chloro-benzophenone in acetone (1 in 20000).

Dissolution Test Perform the test with 1 capsule of Chlordiazepoxide Hydrochloride Capsules at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of water as a dissolution solution. Take the dissolved solution after 30 minutes from the beginning of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Pipet *V* mL of the filtrate accurately, add water to make the concentration of about 6 μg per mL with the volumes of *V'* mL and use this solution as the test solution. Separately, weigh accurately 30 mg of Chlordiazepoxide Hydrochloride RS and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 245 nm, using water as the blank, as directed under Ultraviolet-visible Spectrophotometry. Remove the contents of 12 Chlordiazepoxide Hydrochloride Capsules as completely as possible with the aid of a current of air, determine the absorbance at the same dilution and in the same manner as for the Capsules and make any necessary modifications.

The dissolution rate of Chlordiazepoxide Hydrochloride Capsules in 30 minutes should be not less than 85 %.

Dissolution rate (%) with respect to the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{90}{C} \times \frac{1}{5}$$

W_S: Amount (mg) of Chlordiazepoxide Hydrochloride RS.

C: Labeled amount (mg) of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$) in 1 capsule.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to following method.

Perform this procedure without exposure of daylight using light-resistant vessels. Transfer the contents of 1 capsule to a 200 mL volumetric flask, dissolve in and dilute with water to volume, and filter, discarding the first 20 mL of the filterate. Dilute a portion of the filtrate quantitatively and step-wise with 0.1N hydrochloric acid to obtain a solution having a concentration of about 6 μg of Chlordiazepoxide Hydrochloride per mL. Dissolve a suitable quantity of Chlordiszeposide Hydrocholride RS, accurately weighed, in 0.1 N hydrochloric acid to obtain a standard solution having a known concentration of about 6 μg per mL. Concomitantly determine the absorbances A_T and A_S of the test solution and the standard solution at the wavelength of

maximum absorbance of about 245nm, with a suitable spectrophotometer, using 0.1 mol/L hydrochloric acid as the blank.

$$\text{Amount (mg) of chlordiazepoxide hydrochloride } (C_{16}H_{14}ClN_3O\cdot HCl) \text{ in 1 capsule} = \frac{T}{D} \times C \times \frac{A_T}{A_S}$$

T : Labeled amount (mg) of chlordiazepoxide hydrochloride in 1 capsule.

D : Concentration (μg/mL) of chlordiazepoxide hydrochloride in the test solution, based on the labeled amount.

C : Concentration (μg/mL) of the standard solution

Assay Perform this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately the contents of not less than about 20 Chlordiazepoxide Hydrochloride Capsules and determine the average weight per capsule. Mix the combined contents and transfer an accurately weighed portion of the powder, equivalent to about 60 mg of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$), to a volumetric flask. Add methanol to make 100 mL, mix and filter and discard the first 15 mL of the filtrate. Pipet 5 mL of the clear filtrate into a volumetric flask and add sulfuric acid in ethanol (1 in 360) to make 100 mL. Pipet 10 mL of this solution into a volumetric flask and dilute with sulfuric acid in ethanol (1 in 360) to make 50 mL and use this solution as the test solution. Dissolve an accurately a portion of Chlordiazepoxide Hydrochloride in methanol to obtain a solution having a known concentration of about 60 mg per mL. Dilute this solution quantitatively and step-wise with sulfuric acid in ethanol (1 in 360) to obtain a standard solution having a known concentration of about 6 μg per mL. Concomitantly determine the absorbances of the test solution and the standard solution at the wavelength of maximum absorbance at about 245 nm, as directed under Ultraviolet-visible Spectrophotometry, using sulfuric acid in ethanol (1 in 360) as the blank. Calculate the absorbances, A_T and A_S, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of chlordiazepoxide hydrochloride } (C_{16}H_{14}ClN_3O\cdot HCl) = 10 \times C \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlordiazepoxide Hydrochloride for Injection

Chlordiazepoxide Hydrochloride for Injection is a preparation for injection which is dissolved before use. Chlordiazepoxide Hydrochloride for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O\cdot HCl$: 336.22).

Method of Preparation Prepare as directed under Injections, with Chlordiazepoxide Hydrochloride.

Description Chlordiazepoxide Hydrochloride for Injection is a white crystalline powder and is odorless.
Chlordiazepoxide Hydrochloride for Injection is soluble in water or in ethanol (95) and practically insoluble in hexane.
Chlordiazepoxide Hydrochloride for Injection is affected by light.

Identification Perform the test, as directed under Chlordiazepoxide Hydrochloride.

pH The pH of the solution containing about 1.0 g of Chlordiazepoxide Hydrochloride for Injection in 100 mL of water is between 2.5 and 3.5.

Purity ***Heavy metals and related substances***— Perform the tests according to Methods (1) and (2), as directed under Chlordiazepoxide Hydrochloride.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 4 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 3.57 EU/1 mg of chordiazepoxidehydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed under Chlordiazepoxide Hydrochloride.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Chlordiazepoxide Powder

Chlordiazepoxide Powder contains not less than 93.0 % and not more than 107.0 % of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$: 299.76).

Method of Preparation Prepare as directed under Powders, with Chlordiazepoxide.

Identification (1) Weigh a portion of Chlordiazepoxide Powder, equivalent to 10 mg of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake and filter. To 5 mL of the filtrate, add 0.1 mol/L hydrochloric acid TS to make 100 mL, determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of Chlordiazepoxide Powder, equivalent to 20 mg of Chlordiazepoxide according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, then filter by suction through a glass filter, evaporate the filtrate with the aid of a current of air to dryness and dry the residue in vacuum at 60 ºC for 1 hour. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 1625 cm^{-1}, 1465 cm^{-1}, 1265 cm^{-1}, 850 cm^{-1} and 765 cm^{-1}.

Purity ***Related substances***—Perform this test without exposure to daylight, using light-resistant vessels, To a portion of Chlordiazepoxide Powder, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97 : 3), shake, centrifuge and use the clear supernatant liquid as the test solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97 : 3) to make exactly 50 mL and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone RS in methanol to make exactly 200 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 25 μL of the test solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Particle Size Distribution Test It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Perform this test without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Powder, equivalent to about 0.1 g of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, add exactly 90 mL of methanol, stopper, shake vigorously for 15 minutes and centrifuge. Pipet 10 mL of the clear supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, P_2O_5, 60 ºC) for 4 hours, dissolve in exactly 10 mL of water and 90 mL of methanol. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of Chlordiazepoxide to that of the internal standard for the test solution and the standard solution, respectably.

$$\text{Amount (mg) of chlordiazepoxide } (C_{16}H_{14}ClN_3O) = \text{Amount (mg) of Chlordiazepoxide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20),

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and 0.02 mol/L monobasic ammonium phosphate TS (7 : 3).

Flow rate: Adjust the flow rate so that the retention time of Chlordiazepoxide is about 5 minutes.

System suirability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, Chlordiazepoxide and internal standard are eluted in this order with the resolution between these peaks neing not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the ratios of the peak area of Chlordiazepoxide to that of internal standard.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlordiazepoxide Tablets

Chlordiazepoxide Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$: 299.76).

Method of Preparation Prepare as directed under Tablets, with Chlordiazepoxide.

Identification (1) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 10 mg of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake and filter. To 5 mL of the filtrate, add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible absorption spectrophotometry: it exhibit the maximum absorption at the wavelength between 244 and 248 nm and the minimum absorption at the wavelength 288 and 292 nm.

(2) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 10 mg of Chlordiazepoxide according to the labeled amount, add 10 mL of ether, shake vigorously and centrifuge. Evaporate 5 mL of the clear supernatant liquid by warming on a water-bath to dryness. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 1625 cm^{-1}, 1465 cm^{-1}, 1265 cm^{-1}, 850 cm^{-1} and 765 cm^{-1}.

Purity ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels, To a portion of powdered Chlordiazepoxide Tablets, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97 : 3), shake, centrifuge and use the clear supernatant liquid as the test solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97 : 3) to make exactly 50 mL and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone RS in methanol to make exactly 200 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 25 μL of the test solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Dissolution Test Perform the test with 1 tablet of Chlordiazepoxide Tablets at 100 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take 30 mL or more of the dissolved solution after 60 minutes from the start of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipette the subsequent V mL, add the 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 3.7 μg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 12 mg of Chlordiazepoxide RS, previously dried in a desiccator for 4 hours (in vacuum, P_2O_5, 60 ºC) and dissolve in the 2nd fluid for dissolution test to make exactly 200 mL. Pipette 3 mL of this solution, add the 2nd fluid for dissolution test to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectably, at 260 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Chlordiazepoxide Tablets in 60 minutes is not less than 70 %.

Dissolution rate (%) with respect to the labeled of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 27$$

W_S: Amount (mg) of Chlordiazepoxide RS.
C: Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test without exposure to daylight, using light-resistant vessels. Weigh accurately a portion of powdered Chlordiazepoxide Tablets, equivalent to about 0.1 g of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, add 10 mL of water and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL and centrifuge. Pipet 10 mL of the clear supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Chloroiazepoxide RS, previously dried in a desiccator (in vacuum, P_2O_5, 60 ºC) for 4 hours, dissolve in 1 mL of water and methanol, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL and use this solution as the standard solution. and proceed as directed in the assay under Chlordiazepoxide Powder.

Amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)

$$= \text{Amount (mg) of Chlordiazepoxide RS} \times \frac{Q_T}{Q_S} \times 10$$

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorhexidine Gluconate Solution

Chlorhexidine Gluconate Solution is a solution of digluconate of chlorhexidine.
Chlorhexidine Gluconate Solution contains not less than 19.0 w/v % and not more than 21.0 w/v % of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10}\cdot 2C_6H_{12}O_7$: 897.76).

Description Chlorhexidine Gluconate Solution is a clear, colorless or pale yellow liquid, is odorless and has a bitter taste.
Chlorhexidine Gluconate Solution is miscible with acetic acid (100) or with water.
One mL of Chlorhexidine Gluconate Solution is miscible with not more than 5 mL of ethanol (99.5) or not more than 3 mL of acetone. A white turbidity is produced by further addition of dehydrated ethanol or acetone.
Chlorhexidine Gluconate Solution is gradually colored by light.

Specific gravity— d_{20}^{20} : 1.06 ~ 1.07.

Identification (1) To 50 μL of Chlorhexidine Gluconate Solution, add 5 mL of methanol, 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is observed.

(2) To 0.5 mL of Chlorhexidine Gluconate Solution, add 10 mL of water and 0.5 mL of copper (II) sulfate TS: a white precipitate is produced. Heat to boiling: the precipitate changes to pale purple.

(3) To 10 mL of Chlorhexidine Gluconate Solution, add 5 mL of water, cool on ice and add 5 mL of sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate by filtration, wash with water, recrystalize from diluted ethanol (7 in 10) and dry at 105 ºC for 30 minutes: the crystals thus obtained melt between 130 ºC and 134 ºC.

(4) Neutralize the filtrate obtained in (3) with 5 mol/L hydrochloric acid TS. To 5 mL of this solution, add 0.65 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydrazine and heat in a water-bath for 30 minutes. After cooling, scratch the inner wall of the vessel with a glass rod to induce crystallization. Collect the crystals, dissolve in 10 mL of hot water, add a small amount of activated charcoal and filter. Cool the filtrate, scratch the inner side of the vessel, collect the formed crystals and dry: the crystals thus obtained melt at about 195 ºC (with decomposition).

pH Add 5.0 mL of Chlorhexidine Gluconate Solution to water to make 100 mL: the pH of this solution is between 5.5 and 7.0.

Purity (1) ***4-Chlroaniline***—To 2.0 mL of Chlorhexidine Gluconate Solution, add water to make exactly 100 mL. Pipet 5 mL of the solution and add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS. Add 0.3 mL of sodium nitrite TS, shake and allow to stand for 2 minutes. Add 4 mL of ammonium sulfamate TS and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N*'-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95) and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution—Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS and add water to make exactly 100 mL. Pipet 5 mL of the solution, add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS and proceed as directed for the preparation of the test solution.

(2) ***Related substances***—Pipet 5.0 mL of Chlorhexidine Gluconate Solution, and add water to make 100 mL. Pipet 5 mL of this solution, transfer to a 25 mL volumetric flask, add the diluent to to make a solution so that each mL contains about 2 mg of chlorhexidine gluconate, and use this solution as the test solution. Pipet 3.0 mL of the test solution, add the diluent to make 100 mL, and use this solution as the standard solution (1). Pipet 2.0 mL of the standard solution (1), add the diluent to make 100 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the total area of the peaks other than chlorhexidine from the test solution is not larger than the peak area of chlorhexidine from the standard solution (1) (3.0 %). Exclude any peak with an area less than the peak area of chlorhexidine from the standard solution (2).

Diluent—Dissolve 27.6 g of sodium dihydrogen phosphate dihydrate in 1500 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 2000 mL.

Operating conditions
Proceed as directed in the operating conditions in the Assay.

Residue on Ignition Not more than 0.1 % (2 g, after evaporation).

Assay Pipet 5.0 mL of Chlorhexidine Gluconate Solution, add water to make exactly 250 mL, pipet 5 mL of this solution, add the diluent to make exactly 250 mL, and use this solution as the test solution. Separately, weigh accurately 100 mg of Chlorhexidine Acetate RS, and add water to make exactly 100 mL. Pipet 5 mL

of this solution, add the diluent to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of chlorhexidine in the test solution and standard solution.

Amount (w/v %) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10}\cdot 2C_6H_{12}O_7$) in Chlorhexidine Gluconate Solution

$$= \frac{897.76}{625.55} \times 0.25 \times C \times \frac{A_T}{A_S}$$

897.76: Molecular weight of chlorhexidine gluconate

625.55: Molecular weight of chlorhexidine acetate

C: Concentration (μg/mL) of chlorhexidine acetate in the standard solution

Diluent—Dissolve 27.6 g of sodium dihydrogen phosphate dihydrate in 1500 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 2000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 239 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of a solution prepared by dissolving 27.6 g of sodium dihydrogen phosphate dihydrate and 10 mL of triethylamine in 1500 mL of water, adjusting the pH to 3.0 with phosphoric acid, and adding water to make 2000 mL, and acetonitrile (70 : 30)

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-9	100	0
9-10	100→45	0→55
10-15	45	55
15-16	45→100	55→0
16 ~ 21	100	0

Flow rate: 1.5 mL/minute

System suitability

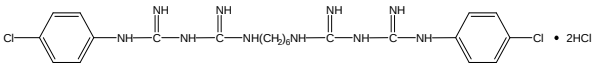
System performance: Dissolve 0.1 mg of Chlorhexidine Acetate RS and 0.1 mg of 4-chloroaniline in 100 mL of the diluent, and use this solution as the system suitability solution. When the procedure is run with 50 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of chlorhexidine and 4-chloroaniline is not less than 3.

System repeatability: When the test is repeated 6 times with 50 μL each of the system suitability solution under the above operating conditions, the relative standard deviations of the peak areas of chlorhexidine and 4-chloroaniline are not more than 2.0 % and not more than 5.0 %, respectively.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorhexidine Hydrochloride



$C_{22}H_{30}Cl_2N_{10}\cdot 2HCl$: 578.37

N-(4-Chlorophenyl)-1-3-(6-{*N*-[3-(4-chlorophenyl)carbamimidamidomethanimidoyl]amino}hexyl) carbamimidamidomethanimidamide hydrochloride [*3697-42-5*]

Chlorhexidine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10}\cdot 2HCl$).

Description Chlorhexidine Hydrochloride is a white, crystalline powder, is odorless and has a bitter taste.

Chlorhexidine Hydrochloride is soluble in formic acid, slightly soluble in methanol or in warm methanol and practically insoluble in water, in ethanol (95) or in ether.

Chlorhexidine Hydrochloride is gradually affected by light.

Identification (1) Dissolve 10 mg of Chlorhexidine Hydrochloride in 5 mL of methanol by warming and add 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is observed.

(2) Dissolve 0.3 g of Chlorhexidine Hydrochloride in 10 mL of 6 mol/L hydrochloric acid TS, cool in ice and add 10 mL of 8 mol/L sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate, wash with water, recrystallize from diluted ethanol (7 in 10) and dry at 105 ºC for 30 minutes: the crystals so obtained melt between 130 ºC and 134 ºC.

(3) Dissolve 0.1 g of Chlorhexidine Hydrochloride in 50 mL of dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Chlorhexidine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with

2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—To 1.0 g of Chlorhexidine Hydrochloride in a crucible, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10), fire the ethanol to burn and heat gradually to incinerate. If a carbonized substance remains, moisten with a small volume of nitric acid and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming in a water-bath, use this solution as the test solution and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 0.2 g of Chlorhexidine Hydrochloride, add mobile phase A to make 100 mL, and use this solution as the test solution. Separately, pipet 3.0 mL of the test solution, add the mobile phase A to make 100 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add mobile phase A to make 100 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions: the total amount of related substances is not more than 3.0 %. Exclude any peak with an area less than the area of the principal peak obtained from the standard solution (2).

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_T}{A_S} \times \frac{C_S}{C_T}$$

C_S: Concentration (mg/mL) of chlorhexidine hydrochloride in standard solution (1)

C_T: Concentration (mg/mL) of chlorhexidine hydrochloride in the test solution

A_T: Peak area of each related substance from the test solution

A_S: Peak area of chlorhexidine from standard solution (1)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 239 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 27.6 g of sodium dihydrogen phosphate dihydrate and 10 mL of triethylamine in 1500 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 2000 mL. To 700 mL of this solution add 300 mL of acetonitrile.

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	100	0
0-15	100	0
15-16	100→45	0→55
16-21	45	55
21-22	45→100	55→0
22-27	100	0

Flow rate: 1.5 mL/minute

(3) **4-*Chloroaniline***—Dissolve 0.10 g of Chlorhexidine Hydrochloride in 2 mL of formic acid and add 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water immediately. Add 0.3 mL of sodium nitrite TS, shake and allow to stand for 2 minutes. Add 4 mL of ammonium sulfamate TS and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylene diamineoxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95) and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution—Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS and add water to make exactly 100 mL. Pipet 5 mL of the solution and add water to make exactly 100 mL. To 2.0 mL of this solution, add 2 mL of formic acid, 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water and proceed in the same manner.

Loss on Drying Not more than 2.0 % (1 g, 130 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

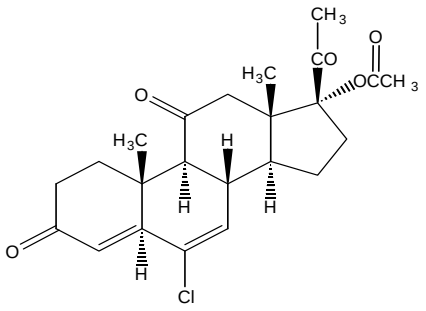
Assay Weigh accurately 0.2 g of Chlorhexidine Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.459 mg of $C_{22}H_{30}Cl_2N_{10}\cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlormadinone Acetate



$C_{23}H_{29}C1O_4$: 404.93

[(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Acetyl-6-chloro-10,13-dimethyl-3-oxo-2,8,9,11,12,14,15,16-octahydro-1*H*-cyclopenta[a]phenanthren-17-yl]acetate [*302-22-7*]

Chlormadinone Acetate, when dried, contains not less than 98.0 % and not more than 101.0 % of chlormadinone acetate ($C_{23}H_{29}C1O_4$).

Description Chlormadinone Acetate appears as white to pale yellow crystals or crystalline powder and is odorless.
Chlormadinone Acetate is freely soluble in chloroform, soluble in acetonitrile, slightly soluble in ethanol (95) or in ether and practically insoluble in water.

Identification (1) Dissolve 2 mg of Chlormadinone Acetate in 1 mL of ethanol (95) and add 1 mL of 1,3-dinitrobenzene TS and 1 mL of a solution of potassium hydroxide (1 in 5): a red-purple color develops.

(2) To 50 mg of Chlormadinone Acetate, add 2 mL of potassium hydroxide-ethanol TS and boil in a water-bath for 5 minutes. After cooling, add 2 mL of diluted sulfuric acid (2 in 7) and boil gently for 1 minute: the odor of ethylacetate is perceptible.

(3) Determine the infrared spectra of Chlormadinone Acetate and Chlormadinone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Chlormadinone Acetate as directed under the Flame Coloration Test (2): a green color appears.

Specific Optical Rotation $[\alpha]_D^{20}$: -10.0 ~ -14.0° (after drying, 0.2 g, acetonitrile, 10 mL, 100 mm).

Melting Point 211 ~ 215 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chlormadinone Acetate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Chlormadinone Acetate according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 20 mg of Chlormadinone Acetate in 10 mL of acetonitrile and use this solution as the test solution. Pipet 1 mL of the test solution, add acetonitrile to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of Chlormadinone Acetate from the test solution is not larger than the peak area of Chlormadinone Acetate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 236 nm).

Column: A stainless steel column, about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 ºC.

Mobile phase: A mixture of acetonitrile and water (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of Chlormadinone Acetate is about 10 minutes.

System suitability

Test for required detection: Pipet 5 mL of the standard solution, add acetonitrile to make exactly 50 mL. The peak area of Chlormadinone Acetate obtained from 10 μL of this solution is between 7 and 13 % of the peak area of Chlormadinone Acetate obtained from 10 μL of the standard solution.

System performance: Dissolve 8 mg of Chlormadinone Acetate and 2 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and chlormadinone acetate are eluted in this order, with the resolution between their peaks being not less than 8.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above conditions: the relative standard deviation of the peak areas of chlormadinone acetate is not more than 1.0 %.

Time span of measurement: About 1.5 times as long as the retention time of chlormadinone acetate after the solvent peak.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 20 mg each of Chlormadinone Acetate and Chlormadinone Acetate

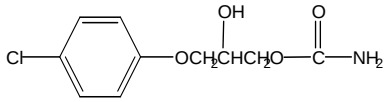
RS, previously dried, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (95) to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry and determine the absorbance, A_T and A_S, at 285 nm, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of chlormadinone acetate } (C_{23}H_{29}ClO_4) = \text{Amount (mg) of Chlormadinone Acetate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorphenesin Carbamate



and enantiomer

$C_{10}H_{12}ClNO_4$: 245.66

[3-(4-Chlorophenoxy)-2-hydroxypropyl]carbamate
[*886-74-8*]

Chlorphenesin Carbamate, when dried, contains not less than 98.0 % and not more than 102.0 % of chlorphenesin carbamate($C_{10}H_{12}ClNO_4$).

Description Chlorphenesin Carbamate appears as white crystals or crystalline powder.
Chlorphenesin Carbamate is freely soluble in methanol, in ethanol (95), or in pyridine, and slightly soluble in water.
A solution of Chlorphenesin Carbamate in ethanol (95) (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Chlorphenesin Carbamate and Chlorphenesin Carbamate RS in ethanol (95) (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chlorphenesin Carbamate and Chlorphenesin Carbamate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Chlorphenesin Carbamate as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 88 ~ 91 °C.

Purity (1) ***Heavy metals***—Dissolve 2.0 g of Chlorphenesin Carbamate in 20 mL of ethanol (95) and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 20 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Chlorphenesin Carbamate according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Chlorphenesin-2-carbamate***—Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of n-hexane for liquid chromatography and 2-propanol(7 : 3) and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area, A_a, of Chlorphenesin Carbamate and the peak area, A_b, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, $A_b/(A_a+A_b)$, is not larger than 0.007.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: At constant temperature.of about 40 °C.

Mobile phase: A mixture of hexane, 2-propanol, and acetic acid (100) (700 : 300 : 1)

Flow rate: Adjust the flow rate so that the retention time of Chlorphenesin Carbamate is about 9 minutes.

System suitability

Test for required detection: Pipet 1 mL of the test solution, add a mixture of hexane for liquid chromatography and 2-propanol (7 : 3) to make 100 mL, and use this solution as the test solution in the system suitability. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is between 40 and 60 % of the peak area of chlorphenesin carbamate obtained from the test solution of the system suitability.

System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in methanol to make 50 mL. To 25 mL of this solution, add 25 mL of dilute sodium hydroxide TS and warm at 60 °C for 20 minutes. To 20 mL of this solution, add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethylacetate, allow to stand to separate the ethyl acetate layer and take the ethyl acetate layer. When the procedure is run with 10 μL of this solution under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2- carbamate are eluted in this order, with the relative retention times of chlorphenesin and chlorphenesin-2-carbamate with respect to

chlorphenesin carbamate being about 0.7 and about 1.2, respectively and with the resolution between the peaks of chlorphenesin and Chlorphenesin Carbamate being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the test solution in the system suitability under the above operation conditions, the relative standard deviation of the peak area of chlorphenesin carbamate is not more than 2.0 %.

(4) ***Other related substances***—Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution and add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 50 μL each of the test solution and the standard solution on a plate of silica gel for liquid chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Allow to stand in iodine vapor for 20 minutes: the number of spots other than the principal spot obtained form the test solution is not more than 1, and this spot is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, in vacuum, silica gel, 4 hours).

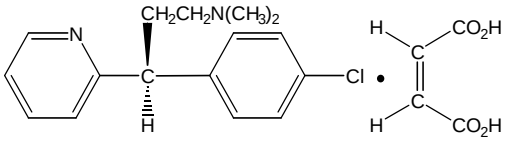
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Chlorphenesin Carbamate, previously dried, dissolve in 20 mL of pyridine, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol VS and warm at 70 °C for 40 minutes. After cooling, add 100 mL of ethanol (95) and titrate the excess potassium hdroxide with 0.1 mol/L hydrochloric acid VS until the color of the solution changes from blue through blue-green to yellow (indicator: 1 mL of thymol blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.566 mg of $C_{10}H_{12}ClNO_4$

Containers and Storage ***Containers***—Tight containers.

Chlorpheniramine Maleate



and enantiomer

$C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86

(*Z*)-but-2-Enedioic acid; [3-(4-chlorophenyl)-3-(pyridin-2-yl)propyl]dimethylamine [*113-92-8*]

Chlorpheniramine Maleate, when dried, contains not less than 98.0 % and not more than 101.0 % of dl-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

Description Chlorpheniramine Maleate appears as white, fine crystals.
Chlorpheniramine Maleate is very soluble in acetic acid (100), freely soluble in water or in methanol, and sparingly soluble in ethanol (95).
Chlorpheniramine Maleate dissolves in dilute hydrocloric acid.
A solution of Chlorpheniramine Maleate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Chlorpheniramine Maleate and Chlorpheniramine Maleate RS in 0.1 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chlorpheniramine Maleate and Chlorpheniramine Maleate RS, previously dried, as directed in the paste method under tInfrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers..

(3) Dissolve 0.1 g of Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the test solution. Separately, dissolve 56 mg of maleinic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot each 5 μL of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ether, methanol, acetic acid (100) and water (70 : 20: 7 : 3) to a distance of about 12 cm and air-dry the plates. And examine the plate under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained from the test solution shows the similar intense and same R_f value with the spot obtained from standard solution.

pH Dissolve about 1.0 g of Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.5.

Melting Point 130 ~ 135 ºC.

Purity (1) ***Clarity and color of solution*** —Dissolve 1.0 g of Chlorpheniramine Maleate in 50 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with1.0 g of Chlorpheniramine Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0

mL of standard lead solution (not more than 20 ppm).,.

(3) ***Related substances***—Dissolve 0.10 g of Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 3 mL of the test solution and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase again to make exactly 20 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions; and determine each peak area of both solutions according to the automatic integration method: the peak area other than the peak of Maleic acid and Chlorpheniramine obtained from the test solution is not larger than 2/3 times the peak area of Chlorpheniramine obtained from the standard solution. The total area of the peaks other than the peaks of maleic acid and chlorpheniramine obtained from the test solution is not larger than the peak area of chlorpheniramine obtained from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 11 minutes.

System suitability

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13 % of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 40000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 4.0 %.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve about 0.4 g of Chlorpheniramine Maleate, previously dried and accurately weighed, in 20 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.543 mg of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorpheniramine Maleate Injection

Chlorpheniramine Maleate Injection is an aqueous solution for injection.

Chlorpheniramine Maleate Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of Preparation Prepare as directed under Injections, with Chlorpheniramine Maleate.

Description Chlorpheniramine Maleate Injection is a clear, colorless liquid.

pH—4.5 ~ 7.0.

Identification Take a volume of Chlorpheniramine Maleate Injection, equivalent to 25 mg of Chlorpheniramine Maleate according to the labeled amount, add 5 mL of dilute sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 10 mL of water, shake with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at 50 ºC under a reduced pressure, anddetermine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Chromatography: it exhibits absorption at the wavenumbers of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 8.8 EU/mg of Chlorpheniramine Maleate.

Foreign Insoluble Matter Test It meets the require-

ment.

Insoluble Particulate Matter for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Transfer an exactly measured volume of Chlorpheniramine Maleate Injection, equivalent to about 3 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), to a separator, add 20 mL of water and 2 mL of sodium hydroxide TS and extract with two 50 mL volumes of ether. Combine the ether extracts, wash with 20 mL of water, and then extract with 20-mL, 20-mL and 5-mL portions of 0.25 mol/L sulfuric acid TS successively. Combine all acid extracts, and add 0.25 mol/L sulfuric acid TS to make exactly 200 mL. Pipet 20 mL of this solution , transfer to a 100-mL separator, add 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of ether. Proceed in the same manner as for the preparation of the test solution, and use the solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and standard solution at a wavelength of the maximum absorbance at about 265 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of chlorpheniramine maleate } (C_{16}H_{19}ClN_2 \cdot C_4H_4O_4) = \text{Amount (mg) of Chlorpheniramine Maleate RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorpheniramine Maleate Powder

Chlorpheniramine Maleate Powder contains not less than 93.0 % and not more than 107.0 % of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of Preparation Prepare as directed under Powders, with Chlorpheniramine Maleate.

Identification Weigh a portion of Chlorpheniramine Maleate Powder, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath under reduced pressure, and determine the spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry: it exhibits absorption at the wavenumber of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately an amount of Chlorpheniramine Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105 ºC for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 µL each of thetest solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of the internal standard.

$$\text{Amount (mg) of chlorpheniramine maleate } (C_{16}H_{19}ClN_2 \cdot C_4H_4O_4) = \text{Amount (mg) of chlorpheniramine maleate RS} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 1.0 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 30 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine after the solvent peak.

Containers and Storage ***Containers***—Tight containers.

Chlorpheniramine Maleate Tablets

Chlorpheniramine Maleate Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of Preparation Prepare as directed under Tablets, with Chlorpheniramine Maleate.

Identification Weigh a portion of powdered Chlorpheniramine Maleate Tablets, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS and filter. Transfer the filtrate to a separator and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes and filter. Evapolate the filtrate in a water bath at about 50 ºC under reduced pressure, and determine the spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry: it exhibits absorption at the wavenumber of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Dissolution Test Perform the test with 1 tablet of Chlorpheniramine Maleate Tablets at 50 revolutions per minute according to Method 2, using 500 mL of 0.01 mol/L hydrochloric acid as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Chlorpheniramine Maleate RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 265 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Chlorpheniramine Maleate Tablets in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 50000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in 1 tablet

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Chlorpheniramine Maleate Tablets add 10 mL of water, shake to disintegrate the tablet, then add water to make exactly V mL of a solution containing about 80 μg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) per mL, and filter through a membrane filter with a pore size of not more than 0.5 um. Pipet 5 mL of the filtrate, add exactly 2.5 mL of internal standard solution, add water to make 10 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105 ºC for 3 hours, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 30 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the conditions described in the Assay, and determine the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of the internal standard.

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$)

$$= \text{Amount (mg) of Chlorpheniramine Maleate RS} \times \frac{Q_T}{Q_S} \times \frac{V}{250}$$

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate (1 in 250) add water to make 1000 mL.

Assay Weigh accurately the mass of not less than 20 Chlorpheniramine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, filter

through a membrane filter with a pore size of not more than 0.5 μm, and use this solution as thetest solution. Separately, weigh acculately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105 ºC for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of this internal standard.

$$\text{Amount (mg) of chlorpheniramine maleate } (C_{16}H_{19}ClN_2.C_4H_4O_4) = \text{Amount (mg) of Chlorpheniramine Maleate RS} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: Astainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: Dissolve 1.0 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability

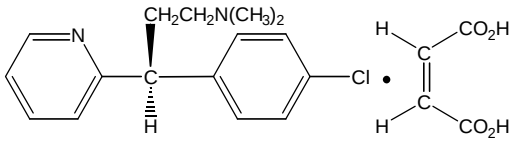
System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 30 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine after the solvent peak.

Containers and Storage ***Containers***—Tight containers.

d-Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86

(*Z*)-but-2-Enedioic acid; (3*S*)-[3-(4-chlorophenyl)-3-(pyridin-2-yl)propyl]dimethylamine [*2438-32-6*]

d-Chlorpheniramine Maleate, when dried, contains not less than 99.0 % and not more than 101.0 % of *d*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

Description *d*-Chlorpheniramine Maleate is a white, crystal powder, is odorless and has a bitter taste.
d-Chlorpheniramine Maleate is very soluble in methanol or in acetic acid (100), and freely soluble in imethylformamide or in ethanol (95).
d-Chlorpheniramine Maleate dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectra of solutions of *d*-Chlorpheniramine Maleate and *d*-Chlorpheniramine Maleate RS in hydrochloric acid TS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths

(2) Determine the infrared spectra of *d*-Chlorpheniramine Maleate and *d*-Chlorpheniramine Maleate RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.1 g of *d*-Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the test solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Deveop the plate with a mixture of ether, methanol, acetic acid (100) and water (70 : 20 : 7 : 3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm), a spot among two of the spots obtained from the test solution shows the same intense to the spot with the stasndard solution, and its R value is about 0.4.

Specific Optical Rotation $[\alpha]_D^{20}$: +39.5 ~ +43.0° (after drying, 0.5 g, dimethylformamide, 10 mL, 100 mm).

Melting Point 111 ~ 115 ºC.

pH Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.0.

Absorbance $E_{1cm}^{1\%}$ (265 nm): 210 ~ 220 (after drying, 5 mg, 0.25 mol/L sulfuric acid TS, 250 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 50 mL of water: the solution is colorless and clear.

(2) ***Heavy metals***—Proceed 1.0 g of *d*-Chlorpheniramine Maleate according to Method 4, and perform the test.Prepare the control solution with 2.0 mL of standard lead solution (not mre than 20 ppm).

(3) ***Related substances***—Dissolve 0.10 g of *d*-Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 3 mL of the test solution, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions; and determine each peak area by the automatic integration method: the peak area other than maleic acid and *d*-chlorpheniramine is not larger than 2/3 times the peak area of *d*-chlorpheniramine obtained from the standard solution, and the total area of the peaks other than maleic acid and *d*-chlorpheniramine is not larger than the peak area of *d*-chlorpheniramine with the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μL in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of *d*-chlorpheniramine is about 11 minutes.

System suitability

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of *d*-chlorpheniramine obtained from 20 μL of this solution is equivalent to 7 to 13 % of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of *d*-chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of *d*-chlorpheniramine is not more than 4.0 %.

Time span of measurement: About 4 times as long as the retention time of *d*-chlorpheniramine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 65 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

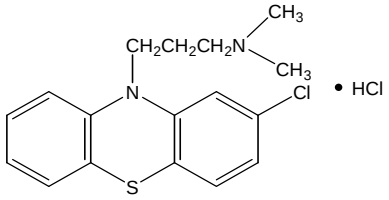
Assay Weigh accurately 0.3 g of *d*-Chlorpheniramine Maleate, previously dried, and dissolve in 20 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.543 mg of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorpromazine Hydrochloride



$C_{17}H_{19}ClN_2S \cdot HCl$: 355.33

3-(2-Chlorophenothiazin-10-yl)-*N,N*-dimethylpropan-1-amine hydrochloride [*69-09-0*]

Chlorpromazine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$).

Description Chlorpromazine Hydrochloride is a white to pale yellow, crystalline powder, is odorless and has a faint, characteristic odor.

Chlorpromazine Hydrochloride is very soluble in water, freely soluble in acetic acid (100) or in ethanol (95), sparingly soluble in acetic anhydride and practically insoluble in ether.

Chlorpromazine Hydrochloride is gradually colored by light.

Identification (1) To 5 mL of a solution of Chlorpromazine Hydrochloride (1 in 1000), add 1 drop of iron (III) chloride TS: a red color is observed.

(2) Dissolve 0.1 g of Chlorpromazine Hydrochloride in 20 mL of water and 3 drops of dilute hydrochloric acid, add 10 mL of 2,4,6-trinitrophenol TS and allow to stand for 5 hours. Collect the resulting precipitate, wash with water, recrystallize from a small volume of acetone and dry at 105 ºC for 1 hour: the crystals so obtained melt between 175 ºC and 179 ºC.

(3) Dissolve 0.5 g of Chlorpromazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS and heat in a water-bath for 5 minutes. Cool, filter and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests (2) for chloride.

Melting Point 194 ~ 198 ºC.

pH Dissolve 1.0 g of Chlorpromazine Hydrochloride in 20 mL of freshly boiled and cooled water and measure within 10 minutes: the pH of this solution is between 4.0 and 5.0.

Purity (1) ***Clarity and color of solution*** —A solution of 1.0 g of Chlorpromazine Hydrochloride in 20 mL of water, when observed within 10 minutes, is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Chlorpromazine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances (other alkylated phenothiazines)***—Weigh accurately 50 mg of Chlorpromazine Hydrochloride, previously dried, dissolve in methanol to make 10 mL, shake, and use this solution as the test solution. Separately, weigh accurately 50 mg of Chlorpromazine Hydrochloride RS, dissolve in methanol to make 10 mL so that each mL contains about 5 mg, and use this solution as the standard stock solution. Pipet 1 mL of the standard stock solution, add methanol to make 20 mL, pipet 1 mL of this solution, add methanol to make 10 mL so that each mL contains 25 μg, and use this solution as the standard solution. Spot 10 μL each of the test solution, standard stock solution, and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, as directed under Thin-layer Chromatography. Develop the plate with a mixture of ether and ammonia solution (28)-saturated ethyl acetate (1 : 1) to a distance of about 10 cm, and air-dry the plate for 20 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the diluted standard solution (not more than 0.5 %).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.7 g of Chlorpromazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.533 mg of $C_{17}H_{19}ClN_2S \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorpromazine Hydrochloride Injection

Chlorpromazine Hydrochloride Injection is an aqueous solution for injection and contains not less than 95.0 % and not more than 105.0 % of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$: 355.33).

Method of Preparation Prepare as directed under Injections, with Chlorpromazine Hydrochloride.

Description Chlorpromazine Hydrochloride Injection is a clear, colorless or pale yellow liquid.

pH—4.0 ~ 6.5.

Identification (1) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 5 mg of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (1) under Chlorpromazine Hydrochloride.

(2) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 0.1 g of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 6.9 EU/mg of chlorpromazine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injection It meets the requirement.

Determination of Volume of Inhection in Containers It meets the requirement.

Assay Transfer an exactly measured volume of Chlorpromazine Hydrochloride Injection, equivalent to about 0.15 g of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$), to a separator, add 30 mL of water and 10 mL of a solution of sodium hydroxide (1 in 5) and extract with two 30 mL volumes and three 20 mL volumes of ether. Wash the combined ether extracts with successive 10 mL volumes of water until the last washing shows no red color upon the addition of phenolphthalein TS. Concentrate the ether extracts on a water-bath to 20 mL, add 5 g of anhydrous sodium sulfate, allow to stand for 20 minutes and filter through a pledget of absorbent cotton. Wash with ether, combine the washings with the filtrate and evaporate the ether on a water-bath. Dissolve the residue in 50 mL of acetone and 5 mL of acetic acid (100) and titrate with 0.05 mol/L perchloric acid VS until the color of the solution changes from red-purple to blue-purple (indicator: 3 drops of bromocresol green methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.767 mg of $C_{17}H_{19}ClN_2S \cdot HCl$

Containers and Storage ***Containers***—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Chlorpromazine Hydrochloride Tablets

Chlorpromazine Hydrochloride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$: 355.33).

Method of Preparation Prepare as directed under Tablets, with Chlorpromazine Hydrochloride.

Identification (1) Shake a portion of powdered Chlorpromazine Hydrochloride Tablets, equivalent to 0.2 g of Chlorpromazine Hydrochloride according to the labeled amount, with 40 mL of 0.1 mol/L hydrochloric acid TS and filter. To 1 mL of the filtrate, add 4 mL of water and 1 drop of ferric chloride TS: a red color is observed.

(2) To 20 mL of the filtrate obtained in (1), add 10 mL of 2,4,6-trinitrophenol TS drop-wise and proceed as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Purity ***Related substances (other alkylated phenothiazines)***—Powder not less than 20 Chlorpromazine Hydrochloride Tablets, weigh accurately a portion of the powder, equivalent to about 50 mg of chlorpromazine hydrochloride according to the labeled amount, transfer to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously, centrifuge, and use as the test solution. In the case of sugar-coated tablets, remove the sugar coating by prior washing with water.Separately, weigh accurately 50 mg of Chlorpromazine Hydrochloride RS, dissolve in methanol to make 10 mL so that each mL contains about 5 mg, and use this solution as the standard stock solution. Pipet 1 mL of the standard stock solution, add methanol to make 20 mL, pipet 1 mL of this solution, add methanol to make 10 mL so that each mL contains 25 μg, and use this solution as the standard solution. Spot 10 μL each of the test solution, standard stock solution, and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, as directed under Thin-layer Chromatography. Develop the plate with a mixture of ether and ammonia solution (28)-saturated ethyl acetate (1 : 1) to a distance of about 10 cm, and air-dry the plate for 20 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the diluted standard solution (not more than 0.5 %).

Dissolution Test Perform the test with 1 tablet of Chlorpromazine Hydrochloride Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of the 2nd fluid for dissolution test as a dissolution solution. Take 20 mL or more of the dissolved solution after 30 minutes from the start of the Dissolution Test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the 2nd fluid for dissolution test to make exactly V' mL so that each mL of the filtrate contains about 5.6 μg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 90 mg of Chlorpromazine Hydrochloride RS, previously dried at 105 ºC for 2 hours, dissolve the 2nd fluid for dissolution test to make exactly 200 mL. Pipet 5 mL of this solution, add the 2nd fluid for dissolution test to make exactly 100 mL, further pipet 5 mL of this solution, add the 2nd fluid for dissolution test to make exactly 20 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 254 nm as directed under Ultraviolet-visible Spectrophotometry.

The dissolution rate of Chlorpromazine Hydrochloride Tablets in 30 minutes should be not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) =

$$W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{45}{8}$$

W_S: Amount (mg) of Chlorpromazine Hydrochloride RS.

C: Labeled amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the procedure without exposure to daylight using the light-resistant vessels. Weigh accurately and powder not less than 20 Chlorpromazine Hydrochloride Tablets. Weigh accurataly a portion of the powder, equivalent to about 50 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S{\cdot}HCl$), add 60 mL of a mixture of dilute phosphoric acid (1 in 500) and dehydrated ethanol, treat with sonicator for 5 minutes, add a mixture (1:1) of dilute phosphoric acid (1 in500) and ethanol (99.5) after mixed with strongly shaking, add a mixture (1:1) of dilute phosphoric aicd (1 in500) and dehydrated ethanol to make exactly 100 mL, filtrate with membrain filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, take the subsequent 2.5 mL, add exactly 5 mL of the internal standard solution, add a mixture (1:1) of dilute with phosphoric acid (1 in500) and ethanol (99.5) to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg of Chlorpromazine Hydrochloride RS, previously dried at 105 ºC for 2 hours, dilute with a mixture (1:1) of dilute phosphoric acid (1 in500) and ethanol (99.5) to make exactly 100 mL. To exactly 5 mL of this solution, add exactly 5 mL of the internal standard solution, dilute with a mixture of dilute phosphoric acid (1 in500) and ethanol (99.5) (1:1) to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. And calculate the ratios, Q_T and Q_S, of the peak area of chlorpromazine hydrochloride to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of chlorpromazine hydrochloride } (C_{17}H_{19}ClN_2S{\cdot}HCl) = \text{Amount (mg) of Chlorpromazine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution ethylparahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1 : 1) (1 in 4500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 30 cm in length, packed with cyano groups chemically bonded silica gel for liquid chromatography (5 μm to10 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (27 : 13).

Flow rate: Adjust the flow rate so that the retention time of Chlorpromazine hydrochloride is about 6.5 minutes.

System suitability

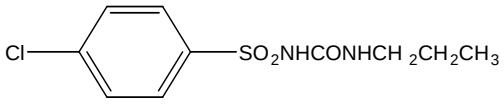
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and chlorpromazine are eluted in this order with the resolution between these peaks the resolution being not less than 10.

System repeatability : When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpromazine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$: 276.74

1-[(4-Chlorobenzene)sulfonyl]-3-propylurea [*94-20-2*]

Chlorpropamide, when dried, contains not less than 98.0 % and not more than 101.0 % of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$).

Description Chlorpropamide appears as white crystals or crystalline powder, and is odorless.
Chlorpropamide is freely soluble in acetone, soluble in ethanol (95), slightly soluble in ether, and practically insoluble in water.

Identification (1) Dissolve 80 mg of Chlorpropamide and Chlorpropamide RS in 50 mL of methanol, then to 1 mL each of these solutions, add 0.01 mol/L hydrochloric acid TS to make 200 mL and determine the absorption spectrum of solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Chlorpropamide and Chlorpropamide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Chlorpropamide as di-

rected under the Flame Coloration Test (2): a green color appears.

Melting Point 127 ~ 131 ºC.

Purity (1) ***Acid***—To 3.0 g Chlorpropamide, add 150 mL of water and warm at 70 ºC for 5 minutes. Allow to stand in ice-water for 1 hour and filter. To 25 mL of the filtrate, add 2 drops of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) ***Chloride***—To 40 mL of the filtrate obtained in (1), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(3) ***Sulfate***—To 40 mL of the filtrate obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Chlorpropamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Selenium***—Proceed with about 0.2 g of Chlorpropamide as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10.0 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(6) ***Related substances***—Dissolve 0.60 g of Chlorpropamide in acetone to make exactly 10 mL and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 300 mL and use this solution as the standard solution (1). Separately, dissolve 60 mg of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 5 µL each of the test solution, the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 3-methyl-1-butanol, methanol and ammonia solution (28) (15 : 10 : 5 : 1) to a distance of about 10 cm and air-dry the plate. After drying the plate at 100 ºC for 1 hour, spray evenly sodium hypochlorite TS on the plate and air-dry for 15 minutes. Then spray evenly potassium iodide-starch TS on the plate: the spot from the test solution equivalent to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2) and the spots other than the spot mentioned above and other than the principal spot from the test solution is not more intense than the spot from the standard solution (1).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

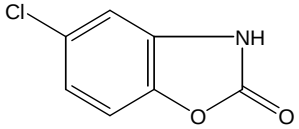
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.5 g of Chlorpropamide, previously dried, dissolve in 30 mL of neutralized ethanol and add 20 mL of water. Titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.674 mg of $C_{10}H_{13}ClN_2O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Chlorzoxazone



$C_7H_4ClNO_2$: 169.57

5-Chloro-2,3-dihydro-1,3-benzoxazol-2-one [*95-25-0*]

Chlorzoxazone contains not less than 98.0 % and not more than 102.0 % of chlorzoxazone ($C_7H_4ClNO_2$), calculated on the dried basis.

Description Chlorzoxazone appears as white to pale yellow crystals or crystalline powder, is odorless, taste-

less and a little irritant.
Chlorzoxazone is freely soluble in *N,N*-dimethylformamide, soluble in methanol, in ethanol (95) or in acetone, slightly soluble in ether and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Chlorzoxazone and Chlorzoxazone RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: the maximum and minimum absorption occurs at the same wavelength.

(2) Determine the infrared spectra of Chlorzoxazone and Chlorzoxazone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 189 ~ 194 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Chlorzoxazone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately a portion of Chlorzoxazone, dissolve in methanol, so that each mL contains 20 mg of Chlorzoxazone and use this solution as the test solution. Separately, weigh accurately a portion of 2-Amino-4-chlorophenol RS, previously dried at 105 ºC for 2 hours and dissolve in methanol so that each mL contains 100 μg and 50 μg of Chlorzoxazone and use these solutions as the standard solutions (1) and (2). Perform the test with the test solution and the standard solutions and (1) and (2) as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and 1,4-dioxane (63 : 37) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (1) (not more than 0.5 %). Also allow to stand in iodine vapor: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (2) (not more than 0.25 %).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

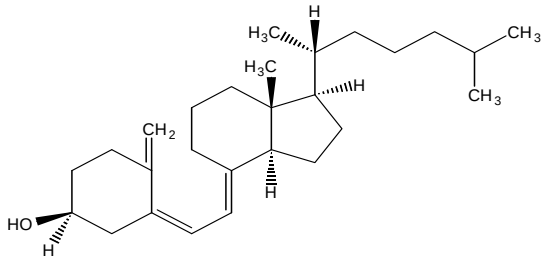
Residue on Ignition Not more than 0.15 % (1 g).

Assay Weigh accurately 50 mg of Chlorzoxazone and Chlorzoxazone RS, previously dried at 105 ºC for 2 hours, dissolve in methanol and dilute with methanol to make exactly 100 mL, respectively. Transfer each 4.0 mL of these solutions to a volumetric flask and dilute with methanol to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with each of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry using methanol as a blank and determine the absorbances at 282 nm, A_T and A_S, of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of chlorzoxazone } (C_7H_4ClNO_2) = \text{Amount (mg) of Chlorzoxazone RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Cholecalciferol



Vitamin D_3 $C_{27}H_{44}O$: 384.65

(1*S*,3*Z*)-3-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(2*R*)-6-methylheptan-2-yl]-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexan-1-ol
[*67-97-0*]

Cholecalciferol contains not less than 97.0 % and not more than 103.0 % of cholecalciferol ($C_{27}H_{44}O$).

Description Cholecalciferol appears as white crystals and is odorless.
Cholecalciferol is freely soluble in ethanol (95), in chloroform, in ether or in isooctane, and practically insoluble in water.
Cholecalciferol is affected by air and by light.

Melting point—84 ~ 88 ºC.
Place Cholecalciferol in a capillary, dry for 3 hours in a desicator (vacuum, not exceeding 2.67 kPa), fire seal the capillary tube immediately, put it in a bath fiuid previously heated to a temperature of about 10 °C beolw the expected melting point, and heat at a rate of rise of about 3 °C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Cholecalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and mix under shaking: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared spectra of Cholecalciferol and Cholecalciferol RS, as directed in the potassium bromide disk method under Infrared

Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +103 ~ +112° (50 mg, ethanol (95), 10 mL, 100 mm). Prepare the solution without delay, using Cholecalciferol from a container opened not longer than 30 minutes previously, and determine the rotation within 30 minutes after the solution has been prepared.

Absorbance $E_{1cm}^{1\%}$ (265 nm): 450 ~ 490 (10 mg, ethanol (95), 1000 mL).

Purity ***7-Dehydrocholesterol***—Dissolve 10 mg of Cholecalciferol in 2.0 mL of diluted ethanol (9 in 10), add a solution prepared by dissolving 20 mg of digitonin in 2.0 mL of diluted ethanol (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Dissolve separately about 30 mg each of Cholecalciferol and Cholecalciferol RS, accurately weighed, in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cholecalciferol to that of the internal standard for the test solution and the standard solutions, respectively. Proceed with the operation avoiding contact with air or other oxidizing agents, and using light-resistant containers.

$$\text{Amount (mg) of cholecalciferol } (C_{27}H_{44}O) = \text{Amount (mg) of Cholecalciferol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 10 to 30 cm in length, packed with silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of hexane and *n*-amylalcohol (997 : 3).

Flow rate: Adjust the flow rate so that the retention time of Cholecalciferol is about 25 minutes.

Selection of column: Dissolve 15 mg of Cholecalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat under a reflux condenser in an oil-bath for 2 hours, and cool to room temperature rapidly. Transfer this solution to a quartz test tube, and irradiate under a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To this solution, add the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D_3, trans-vitamin D_3, and tachysterol D_3 to that of cholecalciferol being about 0.5, about 0.6, and about 1.1, respectively, and with resolution between previtamin D_3 and trans-vitamin D_3, and that between cholecalciferol and tachysterol D_3 being not less than 1.0.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Chorionic Gonadotrophin

Gonadotropin, chorionic [*9002-61-3*]

Chorionic Gonadotrophin is dried preparation of gonad-stimulating hormone obtained from the urine of pregnant women through the removal of the virus or non-active process. Chorionic Gonadotrophin contains not less than 2500 chorionic gonadotrophin units per mg. Chorionic Gonadotrophin contains not less than 80.0 % and not more than 125.0 % of the labeled amount of Chorionic Gonadotrophin.

Description Chorionic Gonadotrophin is a white to pale yellow-brown powder.
Chorionic Gonadotrophin is odorless.
Chorionic Gonadotropin is freely soluble in water and practically insoluble in ether.

Identification Calculate *b* by the following equation, using Y_3 and Y_4 obtained in the Assay: *b* is less than 120.

$$b = \frac{E}{I}$$

$$E = \frac{Y_3 - Y_4}{f}$$

f: Number of test animals per group.

$$I = \log \frac{T_H}{T_L}$$

Purity (1) ***Clarity and color solution***—Dissolve 0.05 g of Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or pale yellow.

(2) ***Estrogen***—Inject subcutaneously into each of

three female albino rats or albino mice ovaectomized at least two weeks before the test, single dose of 100 units according to the labeled units dissolved in 0.5 mL of isotonic sodium chloride solution. Take vaginal smear twice daily, on the third, fourth and fifth day. Place the smear thinly on a slide glass, dry, stain with Giemsa's TS, wash with water and again dry: no estrus figure is shown microscopically.

Loss on Drying Less than 5.0 % (0.1 g, in vacuum, P_2O_5, 4 hours).

Bacterial Endotoxins Less than 0.03 EU/unit of Chorionic Gonadotrophin.

Abnormal Toxicity Weigh the suitable amount of Chorionic Gonadotrophin, prepare a solution containing 120 units per each mL, and use this solution as the test solution. Inject 5.0 mL of the test solution into the peritoneal cavity of each of two or more of, well nourished, healthy guinea pigs weighing about 350 g and observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

Specific Activity When calculate from the results obtained by the Assay and the following test, the specific activity is not less than 3000 human chorionic gonadotrophin Units per mg protein

(1) ***Test solution***—To an exactly amount of Chorionic Gonadotrophin add water to make a solution so that each mL contains about 500 Units of chorionic gonadotrophin according to the labeled amount

(2) ***Standard solution***—Weigh accurately about 10mg of bovine serum albumin, and dissolve in water to make exactly 20mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50μg of the albumin per mL, respectively.

(3) ***Procedure***—Pipet 0.5mL each of the test solution and the four standard solutions, put them in glass test tubes about 18mm in internal diameter and about 130 mm in length, add exactly 5mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30 °C for 10 minutes. Then add exactly 0.5mL of diluted Folin's TS (1 in 2), mix, and warm in a water bath at 30 °C for 20 minutes. Determine the absorbances of these solutions at 750nm as directed under Ultraviolet-visible Spectrophotometry using a solution obtained in the same manner with 0.5mL of water as the blank.
Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and determine the protein content of the test solution from its absorbance by using this curve. Then calculate the amount of the protein in the sample.

Assay (1) ***Test animals***—Select healthy female albino rats weighing about 45 g.

(2) ***Standard solution***—Dissolve a quantity of Chorionic Gonadotrophin RS in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, having 7.5, 15, 30 and 60 units per 2.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each and weigh their ovaries, as directed in procedure of (iv). Separately, inject bovine serum albumin-isotonic sodium chloride solution to one group and use this group as the control group. According to the result of this test, designate the concentration of the RS which will increase the weights of the ovaries about 2.5 times the weight of the ovaries of the control group as a low-dose concentration of the standard solution and the concentration 1.5 to 2.0 times the low-dose concentration as a high dose concentration. Dissolve a quantity of chorionic gonadotrophin RS in bovine serum albumin-isotonic sodium chloride solution and prepare a high-dose standard solution, S_H and a low-dose standard solution, S_L, whose concentrations are equal to those determined by the above test.

(3) ***Test solution***—According to the labeled units, weigh accurately a suitable quantity of Chorionic Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution and prepare a high-dose sample solution, T_H and a low-dose test solution, T_L, having units equal to the standard solutions in equal volumes, respectively.

(4) ***Procedure***—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H, T_L in each group for 5 days. On the sixth day, excise the ovaries and remove the adhering water by lightly pressing between filter paper and immediately weigh the ovaries.

(5) ***Calculation***—Designate the weight of ovaries by S_H, S_L, T_H, T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

$$\text{Units per mg of Chorionic Gonadotrophin} = \text{antilog M} \times (\text{units per mL of the high dose of the standard solution}) \times \frac{b}{a}$$

$$M = \frac{IY_a}{Y_b}$$

$$I = \log\frac{S_H}{S_L} = \log\frac{T_H}{T_L}$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Mass (mg) of sample,

b: Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution.

F' compared by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If F' exceeds F_1, or if L exceeds 0.3, repeat the test increasing the number of the test animals ro arranging the assay method in a better way until F' is smaller than F or L is not more than 0.3.

$$F' = \frac{(Y_1 - Y_2 - Y_3 + Y_4)^2}{4fs^2}$$

F: Number of test animals per group.

$$s^2 = \frac{\sum y^2 - \frac{Y}{f}}{n}$$

$\sum y^2$: The sum of the squares of each y_1, y_2, y_3 and y_4.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f-1)$$

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

t^2: Value shown in the following tablet against n used to calcuate s^2.

n	$t^2=F_1$	n	$t^2=F_1$	n	$t^2=F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Chorionic Gonadotrophin for Injection

Chorionic Gonadotrophin for Injection is a preparation for injection which is reconstituted before use. Chorionic Gonadotrophin for Injection contains not less than 80.0 % and not more than 125.0 % of the labeled units of Chorionic Gonadotrophin.

Method of Preparation Prepare as directed under Injections, with Chorionic Gonadotrophin.

Description Chorionic Gonadotrophin for Injection is a white to pale yellow-brown powder or masses. Chorionic Gonadotropin is freely soluble in water.

Identification Proceed as directed in the Identification under Chorionic Gonadotrophin.

Loss on Drying Not more than 5.0 % (1.0 g, in vacuum, P_2O_5, 4 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.03 EU/mL of Chorionic Gonadotrophin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

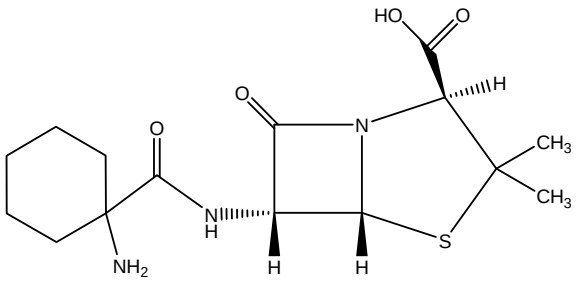
Assay Proceed as directed in the Assay under Chorionic Gonadotrophin. The ratio of the assayed units to the labeled units should be calculated by the following equation.

The ratio of the assayed units to the labeled units
= antilog M

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant, and in a cold place.

Ciclacillin



$C_{15}H_{23}N_3O_4S$: 341.43

(3*S*,5*R*,6*R*)-6-{[(1-Aminocyclohexyl)carbonyl]amido}-2,2-dimethylpenam-3-carboxylic acid [*3485-14-1*]

Ciclacillin contains not less than 920 μg (potency) and not more than 1010 μg (potency) per mg of ciclacillin ($C_{15}H_{23}N_3O_4S$), calculated on the anhydrous basis.

Description Ciclacillin appears as white to pale yellowish white crystalline powder.
Ciclacillin is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in acetonitrile or in ethanol (99.5).

Identification Determine the infrared spectra of Ciclacillin and Ciclacillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +300 ~ +315° (2 g, water, 100 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Ciclacillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Ciclacillin according to Method 3, and perform the test (not more than 2 ppm).

Water Not more than 2.0 % (1 g, volumetric titration, direct titration)

Assay Weigh accurately about 50 mg (potency) each of Ciclacillin and Ciclacillin RS, dissolve each in the mobile phase, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ciclacillin to that of the internal standard.

$$\text{Amount [μg (potency)] of ciclacillin } (C_{15}H_{23}N_3O_4S) = \text{Amount [μg (potency)] of Ciclacillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of orcin in the mobile phase (1 in 500)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 100 mL. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.

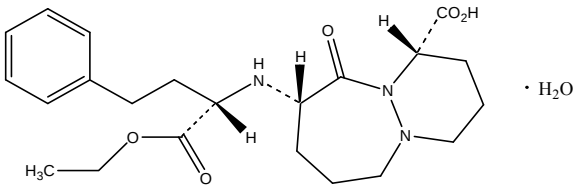
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ciclacillin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Cilazapril Hydrate



$C_{22}H_{31}N_3O_5,H_2O$: 435.51

(1S,9S)-9-{[(2S)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-10-oxo-octahydro-1H-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate
[*92077-78-6*]

Cilazapril Hydrate contains not less than 98.5 % and not more than 101.5 % of cilazapril ($C_{22}H_{31}N_3O_5$), calculated on the anhydrous basis.

Description Cilazapril Hydrate appears as white to pale yellowish white crystals or crystalline powder.
Cilazapril Hydrate is very soluble in methanol, freely soluble in ethanol (99.5) or in acetic acid (100), and slightly soluble in water.
Cilazapril Hydrate is gradually colored to yellow by light.

Identification (1) To 4 mL of a solution of Cilazapril Hydrate (1 in 1000) add 2 mL of Dragendorff's TS: an orange precipitate is produced.

(2) Determine the infrared spectra of Cilazapril Hydrate and Cilazapril Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_{365nm}^{20}$: −383 ~ −399° (0.2 g calculated on the anhydrous basis, 0.067 mol/L

phosphate buffer solution at pH 7.0, 50 mL, 100 mm).

Purity (1) ***Chloride***—Perform the test using 1.0 g of Cilazapril Hydrate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid TS (not more than 0.009 %).

(2) ***Sulfate***—Dissolve 1.0 g of Cilazapril Hydrate in 40 mL of water and 1.5 mL of dilute hydrochloric acid, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution with 0.4 mL of 0.005 mol/L sulfuric acid TS (not more than 0.019 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Cilazapril Hydrate according to Method 4, and perform the test. Use 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 8). Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substance I***—Dissolve 0.20 g of Cilazapril Hydrate in methanol to make 5 mL, and use this solution as the test solution. Separately, dissolve 2 mg of cilazapril related substance I RS {1,1-dimethylethyl(1*S*,9*S*)-9-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-α][1,2]diazepine-1-carboxylate}, accurately weighed, in methanol to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5 mg of cilazapril hydrate related substance I RS and 5 mg of Cilazapril Hydrate in methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution , the standard solution (1), and the standard solution (2) on a plate of silica gel for thin-layer chromatography, develop with a mixture of ethyl acetate, methanol, hexane, acetic acid, and water (60 : 15 : 15 : 5 : 5) to a distance of about 10 cm, and dry the plate in air. Spray the plate first with a freshly prepared mixture of 12 w/v % acetic acid and potassium iodobismuthate solution (10:1) and then with hydrogen peroxide TS. Any spot, corresponding to the related substance I, from the test solution is not more intense than the spot from the standard solution (1) (0.1 %). The test is valid when the chromatogram obtained with the standard solution (2) shows 2 clearly separated spots.

(5) ***Other related substances***—Dissolve 25 mg of Cilazapril Hydrate, accurately weighed, in the mobile to make 50 mL, and use this solution as the test solution. Dilute 1.0 mL of the test solution to 50 mL with the mobile phase. Dilute 5.0 mL of this solution to 20 mL with the mobile phase, and use this solution as the standard solution (1). Separately, dissolve 10 mg of cilazapril hydrate related substance IV RS {(1*S*,9*S*)-9-[[(*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxoocta hydro-6*H*-pyridazino-[1,2-α][1,2]diazepine-1-carboxylic acid}in the test solution to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions. In the chromatogram obtained with the test solution: the area of any peak corresponding to the related substance IV is not greater than 0.4 times the area of the principal peak from the standard solution (1) (0.2 %); the area of any peak corresponding to cilazapril hydrate related substance II {(1*S*,9*S*)-9-[[(*S*)-1-carboxy-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-α][1,2]diazepine-1-carboxylic acid} is not greater than the area of the principal peak from the standard solution (1) (0.5 %); the area of any peak corresponding to cilazapril hydrate related substance III {ethyl(1*S*,9*S*)-9-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-α][1,2]diazepine-1-carboxylate} is not greater than 0.2 times the area of the principal peak from the standard solution (1) (0.1 %); the area of any peak, apart from the principal peak and the peaks corresponding to the related substances II, III and IV, is not greater than 0.2 times the area of the principal peak from the standard solution (1) (0.1 %); and the sum of the areas of all the peaks, apart from the principal peak, is not greater than twice the area of the principal peak from the standard solution (1) (1 %). Disregard any peak with an area less than 0.1 times that of the principal peak from the standard solution (1) and any peak due to the related substance I.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Mix 10 mL of triethylamine and 750 mL of water, adjust to pH 2.30 with phosphoric acid, and add 200 mL of tetrahydrofuran.

Flow rate: 1 mL/min.

System suitability

Test for required detectability: Perform the test with 20 μL each of the test solution and the standard solution (1) according to the above operating conditions. The retention times relative to cilazapril are about 0.6 for the related substance II, 0.9 for the related substance IV, and 1.6 for the related substance III.

System performance: Perform the test with 20 μL each of the standard solutions (1) and (2) according to the above operating conditions. Adjust the sensitivity of the system so that the height of the principal peak from the standard solution (1) is at least 50 % of the full scale of the recorder. The test is valid when the resolution between the peaks of cilazapril and the related substance IV from the standard solution (2) is at least 2.5.

Time span of measurement: 2 times as long as the retention time of cilazapril. When the related substance I with a relative retention time of 4 to 5 is present, the chromatography should be continued until it is eluted.

Water 3.5 ~ 5.0 % (0.30 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

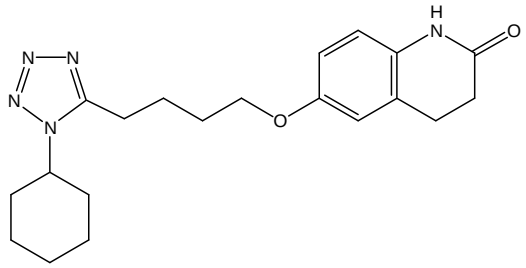
Assay Dissolve 0.30 g of Cilazapril Hydrate, accurately weighed, in 10 mL of ethanol (95) and add 50 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 41.75 mg of $C_{22}H_{31}N_3O_5$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cilostazol



$C_{20}H_{27}N_5O_2$: 369.46

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butyloxy]-3,4-dihydroquinolin-2(1*H*)-one [*73963-72-1*]

Cilostazol, when dried, contains not less than 98.5 % and not more than 101.5 % of cilostazol ($C_{20}H_{27}N_5O_2$).

Description Cilostazol appears as white to pale yellowish white crystals or crystalline powder.
Cilostazol is slightly soluble in methanol, in ethanol (99.5) or in acetonitrile, and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Cilostazol and Cilostazol RS in methanol (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cilostazol and Cilostazol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting point 158 ~ 162 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Cilostazol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 25 mg of Cilastozol in 25 mL of actonitrile, and use this solution as the test solution. Pipet 1 mL of the test solution, and add actonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilostazol obtained with the test solution is not larger than 0.7 times the peak area of cilostazol with the standard solution, and the total area of the peaks other than the peak of cilostazol with the test solution is not larger than 1.2 times the peak area of cilastazol with the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4.6 mm in internal diameter and about 150 mm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of hexane, ethylacetate and methanol (10 : 9 : 1).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 7 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of cilastazol obtained with 10 μL of this solution is equivalent to 7 to 13 % of that with 10 μL of the standard solution.

System performance: Pipet 1 mL of the test solution, add 1 mL of a solution prepared by dissolving 5 mg of 3,4-dihydro-6-hydroxy-2(1*H*)-quinolinone in 10 mL of acetonitrile and acetonitrile to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, 3,4-dihydro-6-hydroxy-2(1*H*)-quinolinone and cilostazol are eluted in this order with the resolution between these peaks being not less than 9.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilostazol is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of cilostazol beginning after the solvent peak.

Loss on Drying Not more than 0.1 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Cilostazol and Cilostazol RS, previously dried, dissolve each in methanol, add exactly 5 ml of the internal standard solution and methanol to make 50 mL. To 1 mL each of these solutions add methanol to make 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of cilostazol to that of the internal standard.

$$\text{Amount (mg) of cilostazol } (C_{20}H_{27}N_5O_2) = \text{Amount (mg) of Cilostazol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzophenone in methanol (1 in 250)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water, acetonitrile and methanol (10 : 7 : 3).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 9 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cilostazol and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Cilostazol Tablets

Cilostazol Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of cilostazol ($C_{20}H_{27}N_5O_2$: 369.46).

Method of Preparation Prepare as directed under Tablests, with Cilostazol.

Identification Mix well an amount of powdered cilostazol Tablets, equivalent to 50 mg of cilostazol according to the labeled amount, with 10 mL of acetone, centrifuge, and use the supernatant liquid as the test solution. Separately, dissolve 25 mg of Cilostazol RS in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 6 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethylacetate, acetonitrile, methanol and formic acid (75 : 25 : 5 : 1) to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the principal spot with the sample solution and the spot with the standard solution are orange in color and have the same R_f value.

Dissolution Test Perform the test with 1 tablet of Cilostazol Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of a solution of sodium lauryl sulfate (3 in 1000) as the dissolution solution. Take 20 mL or more of the dissolved solution 45 minutes after the start of the test for a 50 mg tablet and 60 minutes after the start of the test for a 100 mg tablet, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the solution of sodium lauryl sulfate (3 in 1000) to make V' mL so that each mL contains about 5.6 mg of cilostazol ($C_{20}H_{27}N_5O_2$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Cilostazol RS (previously dried at 105 °C for 2 hours), and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add the solution of sodium lauryl sulfate (3 in 1000) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrometry using the solution of sodium lauryl sulfate (3 in 1000) as the control: the dissolution rates of a 50 mg tablet in 45 minutes and a 100 mg tablet in 60 minutes are not less than 75 % and not less than 70 %, respectively.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cilostazol } (C_{20}H_{27}N_5O_2) = \text{Amount (mg) of Cilostazol RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

C: Labeled amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Cilostazol Tablets. Weigh accurately a portion of the powder, equivalent ot about 50 mg of cilostazol ($C_{20}H_{27}N_5O_2$), add exactly 5 ml of the internal standard solution and methanol to make 50 mL, and shake well for 10 minutes. To 1 mL of this solution add methanol to make 10 mL, filter through a membrane filter with a pore size of not exceeding 0.5 μm, and use the filtrate as the test solution. Separtely, weigh accurately about 50 mg of Cilostazol RS, dissolve in methanol, and add exactly 5 ml of the internal standard solution and methanol to make 50 mL. To 1 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cilostazol to that of the internal standard.

$$\text{Amount (mg) of cilostazol } (C_{20}H_{27}N_5O_2) = \text{Amount (mg) of Cilostazol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzophenone in methanol (1 in 250)

Operating conditions

Proceed as directed in the operating conditions in the Assay under Cilostazol.

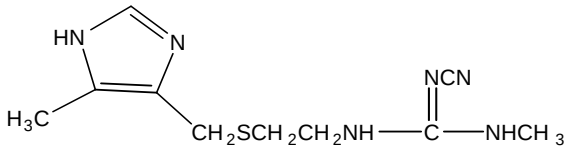
System suitability

System performance: Proceed as directed in the system suitability in the Assay under Cilostazol.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Well-closed containers.

Cimetidine



$C_{10}H_{16}N_6S$: 252.34

2-Cyano-1-methyl-3-{2-[(5-methyl-1*H*-imidazol-4-yl)methylsulfanyl]ethyl}guanidine [*70059-30-2*]

Cimetidine, when dried, contains not less than 99.0 % and not more than 101.0 % of cimetidine ($C_{10}H_{16}N_6S$).

Description Cimetidine is a white crystalline powder, is odorless and has a bitter taste.

Cimetidine is freely soluble in methanol or in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in water and practically insoluble in ether.

Cimetidine dissolves in dilute hydrochloric acid.

Cimetidine is gradually colored by light.

Identification (1) To 0.1 mL of a solution of Cimetidine in ethanol (95) (1 in 100), add 5 mL of citric acid-acetic anhydride TS and heat in a water bath for 15 minutes: a red-purple color develops.

(2) Determine the infrared spectra of Cimetidine and Cimetidins RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 0.5 g of Cimetidine in 50 mL of freshly boiled and cooled water, shake for 5 minutes and filter: the pH of the filtrate is between 9.0 and 10.5.

Melting Point 140 ~ 144 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cimetidine in 10 mL of methanol: the solution is clear and colorless to pale yellow in color.

(2) ***Heavy metals***—Proceed with 2.0 g of Cimetidine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Dissolve 1.0 g of Cimetidine in 5 mL of dilute hydrochloric acid and perform the test with this solution (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.5 g of Cimetidine in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and pipet 1 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 4 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the mixture of ethyl acetate, methanol and ammonia solution (28) (21 : 2 : 2) to a distance of about 15 cm, air-dry the plate and then dry at 80 °C for 30 minutes. Allow the plate to stand in iodine vapor for 45 minutes: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.25 % (1 g).

Assay Weigh accurately about 0.24 g of Cimetidine, previous dried, dissolve in 75 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potenti-

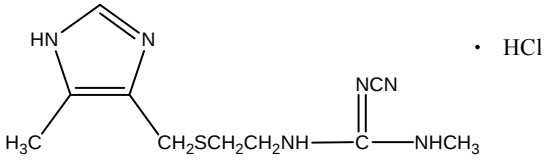
ometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.234 mg of $C_{10}H_{16}N_6S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cimetidine Hydrochloride



$C_{10}H_{16}N_6S$·HCl: 288.80

2-Cyano-1-methyl-3-{2-[(5-methyl-1*H*-imidazol-4-yl)methylsulfanyl]ethyl}guanidine hydrochloride [*70059-30-2*]

Cimetidine Hydrochloride contains not less than 98.0 % and not more than 102.0 % of cimetidine hydrochloride ($C_{10}H_{16}N_6S$·HCl), calculated on the dried basis.

Description Cimetidine Hydrochloride is a white crystalline powder.
Cimetidine Hydrochloride is freely soluble in water, and sparingly soluble in ethanol (95).

Identification (1) Dissolve about 15 mg, accurately weighed, of Cimetidine Hydrochloride and Cimetidine Hydrochloride RS, respectively, in 0.05 mol/L sulfuric acid TS to exactly 100 mL. Pipet 5.0 mL each of the solutions, add 0.05 mol/L sulfuric acid TS to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cimetidine Hydrochloride and Cimetidine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cimetidine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately 0.1 g of Cimetidine Hydrochloride, add mobile phase to make exactly 250 mL, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add mobile phase to make exactly 500 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test and the standard solutions as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method and calculate the percentage of each related substance: Each related substance is not greater than 0.2 %, and the sum of all related substances is not more than 1.0 %.

$$\text{Amount (\%) of each related substance} = 0.2 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance in the chromatogram obtained from the test solution

A_S: Peak area of cimetidine in the chromatogram obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 0.94 g of sodium 1-hexanesulfonate in 240 mL of methanol, and add 0.3 mL of phosphoric acid and water to make 1000 mL.

Flow rate: 2 mL/minute

System suitability

System performance: Dissolve 50 mg of Cimetidine Hydrochloride in 100 mL of 1 mol/L hydrochloric acid TS and heat on a steam bath for about 10 minutes and cool. Pipet 1.0 mL of this solution and add mobile phase to make 250 mL. When the procedure is run with 50 μL of this solution under the above operating condition, the resolution between the peaks of cimetidine and its amide analog is not less than 4.0. And when the procedure is run with 50 μL of the standard solution under the above operating condition, the capacity factor is not less than 3.0 and the number of theoretical plates is not less than 2000.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cimetidine is not more than 7.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.115 g of Cimetidine Hydrochloride, add 50 mL of water to dissolve, add 50 mL of methanol and water to make exactly 250 mL. Pipet 5.0 mL of this solution, add mobile phase to make exactly 200 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of

Cimetidine Hydrochloride RS, add a mixture of water and methanol (80 : 20) to make exactly 100 mL. Pipet 5.0 mL of this solution, add mobile phase to make exactly 200 mL, and use this solutions the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak area of cimetidine, A_T and A_S, respectively.

$$\text{Amount (mg) of Cimetidine Hydrochloride } (C_{10}H_{16}N_6S\cdot HCl) = \text{Cimetidine Hydrochloride RS (mg)} \times \frac{A_T}{A_S} \times 2.5$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: To 200 mL of methanol add 0.3 mL of phosphoric acid and water to make 1000 mL.

Flow rate: about 2 mL/minute

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating condition, the number of theoretical plates is not less than 1000 and capacity factor is not less than 0.6.

System repeatability: When the test is repeated 5 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cimetidine is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cimetidine Tablets

Cimetidine Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$: 252.34).

Method of Preparation Prepare as directed under Tablets, with Cimetidine.

Identification The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation as obtained in the Assay.

Dissolution Test Perform the test with 1 tablet of Cimetidine Tablets at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid as a dissolution solution. Take the dissolved solution 15 minutes after start of the test and make any necessary dilute in 0.01 mol/L hydrochloric acid and use this solution as the test solution. Separately, weigh accurately sufficient quantity of Cimetidine RS and dissolve in 0.01 mol/L hydrochloric acid and make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and standard solution at the maximum absorption wavelength of about 218 nm as directed under Ultraviolet-visible Spectrophotometry, using 0.01 mol/L hydrochloric acid as the blank.

The dissolution rate of Cimetidine Tablets in 15 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Cimetidine Tablets. Weigh accurately a portion of the powder, equivalent to 0.1 g of cimetidine ($C_{10}H_{16}N_6S$), add 50 mL of methanol, shake, mix for 2 minutes, then add 40 mL of water, ultrasonicate, mix for 15 minutes and add water to make exactly 250 mL. Pipet 5.0 mL of this solution and add the mobile phase to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately 20 mg of Cimetidine RS, dissolve in a mixture of water and methanol (4 : 1) to make exactly 50 mL, pipet 5.0 mL of this solution and add mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, A_T and A_S, of the peak area of Cimetidine, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of cimetidine } (C_{10}H_{16}N_6S) = \text{Amount(mg) of Cimetidine RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: To 200 mL of methanol, add 0.3 mL of phosphoric acid , add water to make 1000 mL and mix.

Flow rate: 2 mL/minute.

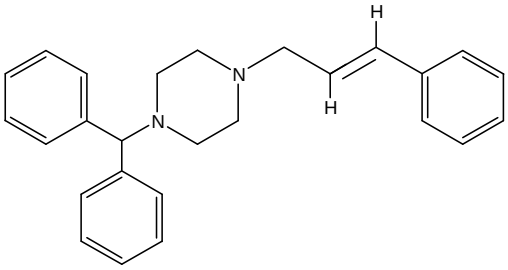
System suitability

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, relative standard deviation of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed

containers.

Cinnarizine



$C_{26}H_{28}N_2$: 368.51

1-Benzhydryl-4-[(*E*)-3-phenylprop-2-enyl]piperazine
[*298-57-7*]

Cinnarizine contains not less than 99.0 % and not more than 101.0 % of cinnarizine ($C_{26}H_{28}N_2$), calculated on the dried basis.

Description Cinnarizine is a white powder.
Cinnarizine is freely soluble in methylene chloride, soluble in acetone, slightly soluble in ethanol (95) or in methanol, and practically insoluble in water.

Identification (1) Dissolve 0.2 g of anhydrous citric acid in 10 mL of acetic anhydride in a water-bath at 80 °C and maintain the temperature for 10 minutes. Add about 20 mg of Cinnarizine. A purple color develops.

(2) Determine the infrared spectra of Cinnarizine and Cinnarizine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 10 mg of Cinnarizine in methanol to make 20 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Cinnarizine RS in methanol to make 5 mL, and use this solution as the standard solution (1). Dissolve 10 mg of Cinnarizine RS and 10 mg of Flunarizine Dihydrochloride RS in methanol and dilute to 20 mL with methanol. Use the solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of acetone, methanol and 1 mol/L sodium chloride solution (50:30:20) to a distance of about 15 cm, and dry the plate in air. Examine in ultraviolet light at 254 nm, and compare the principal spot from the test solution with that from the standard solution (1). Their R_f values are the same. This test is valid when the chromatogram from the standard solution (2) shows two clearly separated spots.

Melting Point 118 ~ 120 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Cinnarizine in methylene chloride to make 20 mL. The solution is clear and has no more color than the following control solution.

Control solution—To a mixture of 24 mL of Iron (III) Cholride Calorimetric Stock Solution, 10 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 4 mL of Cupric Sulfate Colorimetric Stock Solution add 62 mL of 1 w/w % hydrochloric acid and mix. To 2.5 mL of this solution add 97.5 mL of 1 w/w % hydrochloric acid and mix.

(2) ***Acidity or alkalinity***—Suspend 0.5 g of Cinnarizine in 15 mL of water. Boil for 2 minutes, cool, and filter. Dilute the filtrate to 20 mL with water, and use this solution as the test solution. To 10 mL of the test solution, add two drops of phenolphthalein TS and 0.25 mL of 0.01 mol/L sodium hydroxide. The solution is pink. When two drops of methyl red TS and 0.25 mL of 0.01 mol/L hydrochloric acid are added to 10 mL of the test solution, the solution is red.

(3) ***Heavy metals***— Dissolve 1.0 g of Cinnarizine in a mixture of acetone and water (85:15). Add dilute hydrochloric acid until dissolution is complete. Dilute to 20 mL with a mixture of acetone and water (85:15), and use this solution as the test solution. To 12 mL of the test solution, add 2 mL of sodium acetate buffer solution (pH 3.5), and mix with 1.2 mL of thioacetamide. Allow to stand still for 2 minutes. Mix 10 mL of diluted lead standard solution with 2 mL of the test solution, proceed in the same manner as above, and use this solution as the standard solution. The color obtained with the test solution is not more intense than that observed with the standard solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 25.0 mg of Cinnarizine in methanol to make exactly 100 mL, and use this solution as the test solution. Separately, dissolve 12.5 mg of Cinnarizine RS and 15.0 mg of Flunarizine Dihydrochloride RS in methanol to make 100 mL. Dilute 1.0 mL of this solution to 20 mL with methanol, and use the solution as the standard solution (1). Dilute 1.0 mL of the test solution to 100 mL with methanol. Dilute 5.0 mL of this solution to 20 mL with methanol, and use this solution as the standard solution (2). Perform the test with 10 μL each of methanol (as a blank), the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions. In the chromatogram of the test solution, the area of any peak, other than the principal peak, is not greater than the area of the principal peak from the standard solution (2) (0.25 %); and the sum of the areas of the peaks, other than the principal peak, is not greater than twice the area of the principal peak from the standard solution (2) (0.5 %). Disregard any peak due to the blank and any peak with an area less than 0.2 times the area of the principal peak from

the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.0 mm internal diameter and 10 cm in length, packed with base-deactivated octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: Variable mixtures of mobile A and mobile phase B, and program the chromatograph as follows.

Mobile phase A: 1 w/v % ammonium acetate solution

Mobile phase B: 0.2 v/v % solution of glacial acetic acid in acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20	75→10	25→90
20-25	10	90
25-30	75	25
30=0	75	25

Flow rate: 1.5 mL/min.

System suitability

System performance: Adjust the sensitivity of the system so that the height of the principal peak is at least 50 % of the full scale of the recorder. When the procedure is run with the standard solution (1) under the above operating conditions, the retention times are about 11 minutes for cinnarizine and about 11.5 minutes for flunarizine. The test is valid when the resolution of these peaks between cinnarizine and flunarizine is at least 5.0. If necessary, adjust the time programm for the gradient elution.

Loss on Drying Not more than 0.5 % (1 g, vacuum, 60 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

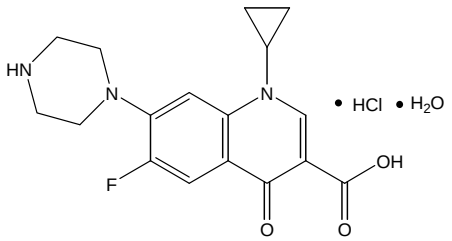
Assay Dissolve 0.15 g of Cinnarizine, accurately weighed, in 50 mL of a mixture of ethyl methyl ketone and acetic acid (100) (7 : 1), and titrate with 0.1 mol/L perchloric acid VS (indicator: 4 drops of naphtholbenzein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.426 mg of $C_{26}H_{28}N_2$.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Ciprofloxacin Hydrochloride Hydrate



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$: 385.82

1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid hydrate hydrochloride [*86393-32-0*]

Ciprofloxacin Hydrochloride Hydrate contains not less than 98.0 % and not more than 102.0 % of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$), calculated on the anhydrous basis.

Description Ciprofloxacin Hydrochloride Hydrate is a pale yellow, crystalline powder.

Ciprofloxacin Hydrochloride Hydrate is sparingly soluble in water, slightly soluble in acetic acid (31) or methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetone, acetonitrile, ethyl acetate, hexane or dichloromethane.

Identification (1) Determine the infrared spectra of Ciprofloxacin Hydrochloride Hydrate and Ciprofloxacin Hydrochloride Hydrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 0.1 g of Ciprofloxacin Hydrochloride Hydrate in water to make 10 mL and use this solution as the test solution. Separately, dissolve 0.1 g of Ciprofloxacin Hydrochloride Hydrate RS in 10 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test and the standard solution on a plate of silica gel for thin-layer chromatography. Allow the plate to stand in the container with ammonia gas for 15 minutes. Develop the plate with a mixture of dichloromethane, methanol, ammonia solution (28) and acetonitrile (4 : 4 : 2 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 365 nm): the spot from the test solution exhibits similar intensity and same R_f value as the spot from the standard solution.

(3) A solution of Ciprofloxacin Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Ciprofloxacin Hydroxide Hydrate in 40 mL of freshly boiled and cooled water: the pH of this solution is between 3.0 and 4.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Ciprofloxacin Hydrochloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Sulfate***—Perform the test with 0.375 g of Ciprofloxacin Hydrochloride Hydrate. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.04 %).

(3) ***Fluoroquinolonic acid***—Weigh a suitable amount of Ciprofloxacin Hydrochloride Hydrate, dissolve in water to make a solution containing 10 mg per mL and use this solution as the test solution. Separately, weigh accurately about 5.0 mg of Fluoroquinolonic Acid RS, add 50 μL of ammonia TS and water to make 50 mL. Pipet 2.0 mL of this solution, add water to make 10 mL and use this solution as the standard solution. Perform the test according to Thin-layer Chromatography with the test solution and the standard solution. Spot 5 μL each of the test and the standard solution on a plate with silica gel for thin-layer chromatography. Allow the plate to stand in the container with 50 mL of ammonia TS for 15 minutes. Develop the plate with a mixture of dichloromethane, methanol, ammonia solution (28) and acetonitrile (4 : 4 : 2 : 1) to a distance of about 15 cm and air-dry the plate for 15 minutes. Examine under the ultraviolet light (main wavelength: 250 nm): R_f value corresponding to the principal spot from the standard solution, the spots other than the principal spot from the test solution are not larger or not more intense than the spot from the standard solution (not more than 0.2 %).

(4) ***Related substances***—Perform the test according to the operating conditions in the Assay. Determine each peak area of related substances, A_i, from the test solution and the total area of all peaks, A_T and calculate the content of each peak area of related substances according to the following equation.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

Each derivative of ciprofloxacin ethylenediamine and related substance is not more than 0.2 % and the total of all related substances is not more than 0.5 %.

Water 4.7 ~ 6.7 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg of Ciprofloxacin Hydrochloride Hydrate, dissolve in mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Ciprofloxacin Hydrochloride Hydrate RS (determined formerly water contents as directed under water determination assay), dissolve in mobile phase to make a solution containing about 0.5 mg per mL and use this solution as the standard solution. Perform the test with 10 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak area of ciprofloxacin hydrochloride, A_T and A_S, of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of ciprofloxacin hydrochloride } (C_{17}H_{18}FN_3O_3\cdot HCl) = 50 \times C \times \frac{A_T}{A_S}$$

C: the concentration of Ciprofloxacin Hydrochloride Hydrate RS in the standard solution, calculated on the anhydrous basis (mg/mL).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column Temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of 0.025 mol/L phosphoric acid adjusted to pH 3.0 ± 0.1 with triethylamine and acetonitrile (87 : 13).

Flow rate: 1.5 mL/minute.

System suitability

System performance: Dissolve Ciprofloxacin Ethylenediamine derivative RS in the standard solution of ciprofloxacin to make a solution containing 0.5 mg per mL. Perform the test with 10 μL of this solution according to the above operating conditions. Ciprofloxacin ethylenediamine derivative and ciprofloxacin are eluted in this order and the resolution between ciprofloxacin ethylenediamine derivate and ciprofloxacin are not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, relative standard derivation of the peak area of ciprofloxacin is no more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ciprofloxacin Hydrochloride Tablets

Ciprofloxacin Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled

amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$: 331.34).

Method of Preparation Prepare as directed under tablets, with Ciprofloxacin Hydrochloride Hydrate.

Identification (1) The retention time of the major peak in the chromatography of the test solution corresponds to that of the standard solution obtained as directed in the Assay.

(2) Place a number of tablets, equivalent to 1.5 g of ciprofloxacin, in a suitable flask containing 750 mL of water and sonicate for about 20 minutes. Dilute with water to 1000 mL and centrifuge a portion of this suspension and use the clear supernatant liquid obtained as the test solution. Separately, dissolve a suitable quantity of Ciprofloxacin Hydrochloride Hydrate RS in water to obtain a solution containing 1.5 mg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Allow the plate to stand in the container with ammonia gas for 15 minutes. Develop the plate with a mixture of dichloromethane, methanol, ammonia TS and acetonitrile (4 : 4 : 2 : 1) to a distance of about 15 cm. Remove the plate and allow it to air-dry for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the principal spot from the test solution exhibits the same R_f value as the principal spot from the standard solution.

Dissolution Test Perform the test with 1 tablet of Ciprofloxacin Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid as a dissolution solution. 30 minutes later after starting the test, take the dissolution solution and filter. If necessary, make a test solution by diluting with the dissolution solution. Separately weigh accurately a portion of ciprofloxacin hydrochloride hydrate and dissolve in the dissolution solution to the same concentration with the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as a blank. The dissolution rate of ciprofloxacin hydrochloride in 30 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay Transfer 5 tablets in 400 mL of water and sonicate for about 20 minutes. Dilute with water to make exactly 500 mL. Dilute an accurately measured volume of this solution with water to obtain a solution containing the equivalent of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) about 0.3 mg per mL and use this solution as the test solution. Separately dissolve a suitable amount, accurately weighed, of Ciprofloxacin Hydrochloride Hydrate RS (formerly determined water contents as directed under water determination assay) in water to obtain a solution containing about 0.3 mg per mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions of the Assay of Ciprofloxacin Hydrochloride Hydrate and determine the peak areas, A_T and A_S, of ciprofloxacin for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of ciprofloxacin } (C_{17}H_{18}FN_3O_3) = C \times \frac{L}{D} \times \frac{A_T}{A_S} \times \frac{331.34}{367.81}$$

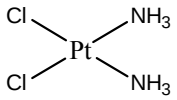
C : Concentration of ciprofloxacin hydrochloride in the standard solution, calculated on the anhydrous basis (mg/mL).

L : Labeled amount of ciprofloxacin in each tablet (mg).

D : Concentration of Ciprofloxacin Hydrochloride in the test solution (mg/mL).

Containers and Storage ***Containers***—Tight containers.

Cisplatin



$Cl_2H_6N_2Pt$: 300.05

(*SP*-4-2)-Diamminedichloridoplatinum [*15663-27-1*]

Cisplatin, when dried, contains not less than 98.0 % and not more than 102.0 % of cisplatin ($Cl_2H_6N_2Pt$).

Description Cisplatin is a yellow crystalline powder. Cisplatin is sparingly soluble in ammonium peroxydisulfate, slightly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Add 2 to 3 drops of tin (II) chloride dihydrate solution (1 in 100) to 5 mL of a solution of Cisplatin in water (1 in 2000): brown precipitate forms.

(2) Determine the absorption spectra of the solutions of Cisplatin and Cisplatin RS, respectively, in a solution of sodium chloride (9 in 1000) in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Cisplatin and

Cisplatin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Cisplain (1 in 2000) responds to the Qualitative Tests (1) for chloride.

Crystallinity Test It meets the requirement.

Purity (1) ***Trichloroammineplatinate***—Perform this procedure without exposure of daylight, using light-resistant ressels. Weigh 50 mg of Cisplatin, and add a solution of sodium chloride (9 in 1000) to make exactly 100 mL and use this solution as the test solution. Separately, weigh 10 mg of Trichloroammineplatinate RS, previously dried at 80 ºC for 3 hours, add a solution of sodium chloride (9 in 1000) to make exactly 200 mL. Pipet 2.0 mL of this solution, add a solution of sodium chloride (9 in 1000) to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 40 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area trichloroammineplatinate from the test solution is not larger than the peak area from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 209 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with quartanaryammonium group introduced octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A solution of ammonium sulfate (1 in 800)

Flow rate: Adjust the flow rate so that the retention time of trichloroammineplatinate is about 8 minutes.

System suitability

System performance: When the procedure is run with 40 μL of the standard solution under the above operating condition, the number of theoretical plates and the symmetry factor of the peak of trichloroammineplatinate are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichloroammineplatinate is not more than 3.0 %.

Loss on Drying Not more than 0.1 % (1 g, 105 °C, 4 hours).

Assay Perform this procedure without exposure of daylight, using light-resistant ressels. Weigh accurately about 25 mg each of Cisplatin and Cisplatin RS, previously dried, add ammonium peroxydisulfate to make exactly 25 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with exactly 40 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine peak area of cisplatin, A_T and A_S, by the automatic integration method, respectively.

$$\text{Amount (mg) of cisplation } (Cl_2H_6N_2Pt) = \text{Amount (mg) of Cisplation RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with aminopropylsilyl silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture solution of ethyl acetate, methanol, water and ammonium peroxydisulfate (25 : 16 : 5 : 5)

Flow rate: Adjust the flow rate so that the retention time of cisplatin is about 4 minutes.

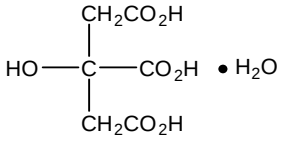
System suitability

System performance: When the procedure is run with 40 μL of the standard solution under the above operating condition, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Citric Acid Hydrate



$C_6H_8O_7 \cdot H_2O$: 210.14

2-Hydroxypropane-1,2,3-tricarboxylic acid hydrate [*5949-29-1*]

Citric Acid Hydrate contains not less than 99.5 % and not more than 100.5 % of anhydrous citric acid ($C_6H_8O_7$: 192.12), calculated on the anhydrous basis.

Description Citric Acid Hydrate appears as colorless crystals, white granule or crystalline powder, is odorless and has a strong acid taste.
Citric Acid Hydrate is very soluble in water, and freely soluble in ethanol (95).
Citric Acid Hydrate is efflorescent in dry air.

Identification Determine the infrared spectra of Citric Acid Hydrate and Citric Acid Hydrate RS, previously dried at 105 ºC for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra similar intensities of absorption at the same wave numbers.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of Citric Acid Hydrate in water to make 10 mL: the solution is clear and has no more color than the following control solutions (1), (2), or (3).

Control solution (1): To 1.5 mL of cobalt (II) chloride hexahydrate colorimetric stock solution and 6.0 mL of iron (III) chloride hexahydrate colorimetric stock solution add water to make 1000 mL.

Control solution (2): To 0.15 mL of cobalt (II) chloride hexahydrate colorimetric stock solution, 7.2 mL of iron (III) chloride hexahydrate colorimetric stock solution and 0.15 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

Control solution (3): To 2.5 mL of cobalt (II) chloride hexahydrate colorimetric stock solution, 6.0 mL of iron (III) chloride hexahydrate colorimetric stock solution and 1.0 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

(2) ***Sulfates***—Dissolve 2.0 g of Citric Acid Hydrate in water to make 30 mL, and use this solution as the test solution. Separately, dissolve 0.181 g of potassium sulfate in diluted dehydrated ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted dehydrated ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the test solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution—Dissolve 0.180 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution and add water to make exactly 100 mL. Proceed with 15 mL of this solution in the same manner as the test solution, and use this solution as the control solution.

(3) ***Oxalic acid***—Dissolve 0.80 g of Citric Acid Hydrate in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the clear supernatant liquid, add 0.25 mL of a solution of phenyl hydrazinium hydrochloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time.

Control solution—To 4 mL of a solution of oxalic acid dihydrate (1 in 10000) add 3 mL of hydrochloric acid and 1 g of zinc.

(4) ***Heavy metals***—Proceed with 2.0 g of Citric Acid Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Readily carbonizable substances***—Perform the test with 0.5 g of Citric Acid Hydrate, provided that the solution is heated at 90 °C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water 7.5 ~ 9.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

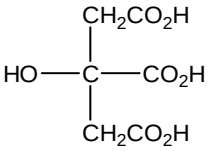
Bacterial Endotoxins Less than 0.5 EU/mg of citric acid hydrate, when Citric Acid Hydrate is used in a non-oral preparation without a further procedure for the removal of bacterial endotoxins.

Assay Weigh accurately about 1.5 g of Citric Acid Hydrate, dissolve in 50 mL of water and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

Containers and Storage ***Containers***—Tight containers.

Anhydrous Citric Acid



$C_6H_8O_7$: 192.12

2-Hydroxypropane-1,2,3-tricarboxylic acid [*77-92-9*]

Anhydrous Citric Acid contains not less than 99.5 % and not more than 101.0 % of anhydrous citric acid ($C_6H_8O_7$), calculated on the anhydrous basis.

Description Anhydrous Citric Acid appears as colorless crystals, white granule or crystalline powder.
Anhydrous Citric Acid is very soluble in water, and freely soluble in ethanol (95).

Identification Proceed as directed in the Identification under Citric Acid Hydrate.

Purity Proceed as directed in the Purity under Citric Acid Hydrate.

Water Not more than 1.0 % (2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

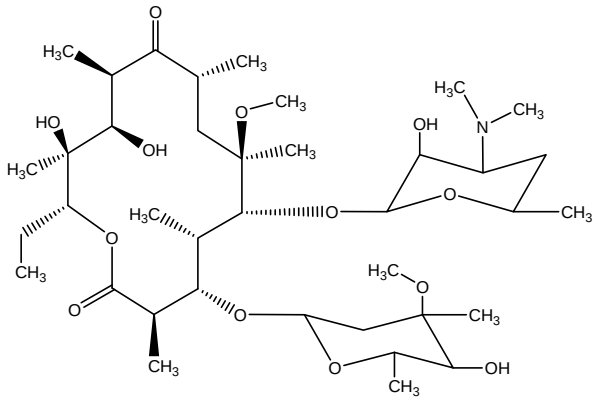
Bacterial Endotoxins Less than 0.5 EU/mg of citric acid, when Anhydrous Citric Acid is used in a non-oral preparation without a further procedure for the removal of bacterial endotoxins.

Assay Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/ L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

Containers and Storage ***Containers***—Tight containers.

Clarithromycin



$C_{38}H_{69}NO_{13}$: 747.95

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione
[*81103-11-9*]

Clarithromycin is a derivative of erythromycin.
Clarithromycin contains not less than 950 μg (potency) and not more than 1050 μg (potency) per mg of clarithromycin ($C_{38}H_{69}NO_{13}$), calculated on the anhydrous basis.

Description Clarithromycin is a white crystalline powder, and has a bitter taste.
Clarithromycin is soluble in acetone or in chloroform, slightly soluble in methanol or in ethanol (95), and practically insoluble in water.

Identification (1) To 5 mg of Clarithromycin add 2 mL of sulfuric acid, and mix with shaking: the red-brown color develops.

(2) To 3 mg of Clarithromycin, add 2 mL of acetone, and add 2 mL of hydrochloric acid: the orange color develops and the color turns immediately to red to deep purple.

(3) Determine the infrared spectra of Clarithromycin and Clarithromycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve separately 10 mg each of Clarithromycin and Clarithromycin RS in 4 mL of chloroform, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, and ammonia solution (28) (100 : 5 : 1) to a distance of about 15 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105 °C for 10 minutes: the spots obtained from the test solution and standard solution are dark purple in color and have the same R_f value.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -81 ~ -97° (0.25 g calculated on the anhydrous basis, chloroform, 25 mL, 100 mm).

Melting Point 220 ~ 227 °C.

pH Weigh 50 mg of Clarithromycin, dissolve in 20 mL of methanol, and take 10 mL of this solution and add water to make 20 mL, and use this solution as the test solution. The pH of the solution is between 8.0 and 10.0.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Clarithromycin according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Clarithromycin according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately 0.1 g of Clarithromycin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Clarithromycin RS, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the amount of each related substance from the test solution calculated on the anhydrous basis is not more than 2.0 %, and the total amount of related substances is not more than 5.0 %. Exclude any peak with an area less than 0.05 %.

$$\text{Amount (\%) of each related substance in Clarithromycin calculated on the anhydrous basis} = \frac{W_S}{W_T} \times \frac{A_i}{A_S} \times 100$$

$$\text{Total amount (\%) of related substances in Clarithromycin calculated on the anhydrous basis} = \frac{W_S}{W_T} \times \frac{\Sigma A_i}{A_S} \times 100$$

W_S: Amount (mg) of Clarithromycin RS taken

W_T: Amount (mg) of Clarithromycin taken, calculated on the anhydrous basis

A_S: Peak area of clarithromycin from the standard solution

A_i: Peak area of each related substance from the test solution

$\sum A_i$: Total area of the peaks other than clarithromycin from the test solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 10 mL, and use this solution as the system suitability solution. Confirm that the peak area of clarithromycin obtained from 10 μL of this solution is equivalent to 14 to 26 % of that from the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of clarithromycin is not more than 3.0 %.

Time span of measurement: About 5 times as long as the retention time of the main peak 2 minutes after injecting the test solution.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (2.0 g).

Assay Weigh accurately about 0.1 g (potency) each of Clarithromycin and Clarithromycin RS, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 2 mL each of these solutions, add 2 mL of the internal standard solution, add the mobile phase to make exactly 20 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clarithromycin to that of the internal standard.

$$\text{Amount [μg (potency)] of clarithromycin } (C_{38}H_{69}NO_{13}) = \text{Amount [μg (potency)] of Clarithromycin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 20000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 50 °C

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) and acetonitrile (13 : 7)

Flow rate: Adjust the flow rate so that the retention time of clarithromycin is about 8 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Clarithromycin Delayed-Release Tablets

Clarithromycin Delayed-Release Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$: 747.96).

Method of Preparation Prepare as directed under Tablets, with Clarithromycin.

Identification (1) Extract an amount of powdered Clarithromycin Delayed-Release Tablets with chloroform so that each mL contains 2.5 mg of clarithromycin, and proceed as directed in the Identification (4) under Clarithromycin.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Dissolution Test Perform the test with 1 tablet of Clarithromycin Delayed-Release Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.21 mol/L phosphate buffer solution (pH 4.0) as the dissolution solution. Take the dissolved solution 2, 8, and 20 hours after the start of the test, and filter. If necessary, dilute with 0.21 mol/L phosphate buffer solution (pH 4.0) so that each mL contains about 560 μg (potency) of clarithromycin according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Clarithromycin RS, dissolve in 7 mL of acetonitrile, add 0.21 mol/L phosphate buffer solution (pH 4.0) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed in the Assay. The dissolution rates of Clarithromycin Delayed-Release Tablets in 2 hours, in 8 hours, and in 20 hours are not less than 15 %, not less than 35 % and not more than 65 %, and not less than 60 %, respectively.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Clarithromycin Delayed-Release Tablets. To a portion of the powder, equivalent to about 0.4 g (potency) according to the labeled potency, add methanol, and shake vigorously to make a solution so that each mL contains 4 mg (potency). Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 40 mg (potency) of Clarithromycin RS, and dissolve in methanol to make a solution so that each mL contains 4 mg (potency). Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatograph according to the following conditions, and determine the peak areas, A_T and A_S, of clarithromyicn in the test solution and standard solution.

Amount [μg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)
= Amount [μg (potency)] of Clarithromycin RS

$$\times \frac{A_T}{A_S} \times 10$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of methanol and 0.067 mol/L potassium dihydrogen phosphate solution (3 : 2) to 3.5 with phosphoric acid.

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Clarithromycin for Syrup

Clarithromycin for Syrup is a preparation for syrup, which is suspended before use.
Clarithromycin for Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$: 747.96).

Method of Preparation Prepare as directed under Syrups, with Clarithromycin.

Identification (1) Proceed as directed in the Identification (4) under Clarithromycin.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH The pH of a solution obtained by dissolving Clarithromycin for Syrup according to the label is between 4.0 and 5.4.

Loss on Drying Not more than 2.0 % (1.0 g, 0.7 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 10 mL of a suspension of Clarithromycin for Syrup, prepared according to the label, at 50 revolutions per minute according to Method 2 at 37 ± 0.5 °C, using 900 mL of 0.05 mol/L potassium dihydrogen phosphate buffer solution (pH 6.8) as the dissolution solution. Take not less than 20 mL of the dissolved solution 45 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subse-

quent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 200 μg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Clarithromycin RS, dissolve in methanol to make exactly 25 mL, pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of clarithromycin. The dissolution rate of Clarithromycin for Syrup in 45 minutes is not less than 80 % of the labeled potency.

$$\text{Dissolution rate (\%) with respect to the labeled amount of clarithromycin } (C_{38}H_{69}NO_{13}) = \frac{\text{Amount [mg (potency)] of Clarithromycin RS}}{W_S} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 360$$

W_S: Amount (g) of Clarithromycin for Syrup in 10 mL of the suspension

C: Labeled amount [mg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$) in 1 g

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of methanol and 0.067 mol/L potassium dihydrogen phosphate (65 : 35) to 4.0 with phosphoric acid.

Flow rate: 1.0 mL/minute

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Clarithromycin Delayed-Release Tablets. Weigh accurately an amount of Clarithromycin for Syrup, equivalent to about 0.5 g (potency), dissolve in 40 mL of water (if necessary, 0.067 mol/L potassium dihydrogen phosphate (pH 6.8)), add methanol to make exactly 100 mL, pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately bout 50 mg (potency) of Clarithromycin RS, dissolve in methanol to make exactly 25 mL, pipet 5 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Clarithromycin Tablets

Clarithromycin Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$: 747.95).

Method of Preparation Prepare as directed under Tablets, with Clarithromycin.

Identification To an amount of powdered Clarithromycin Tablets, equivalent to 60 mg (potency) of clarithromycin according to the labeled amount, add 40 mL of acetone, shake for 10 minutes, and centrifuge at 4000 revolutions per minute for 5 minutes. Evaporate 30 mL of the clear supernatant liquid, and determine the infrared spectrum of the residue thus obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 2980 cm^{-1}, 2940 cm^{-1}, 1734 cm^{-1}, 1693 cm^{-1}, 1459 cm^{-1}, 1379 cm^{-1}, and 1171 cm^{-1}.

Water Not more than 7.0 % (0.1 g, volumetric titration, direct titration).

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the
Content uniformity test.

To 1 tablet of Clarithromycin Tablets add exactly V/20 mL of the internal standard solution (1), then add the mobile phase to make V mL so that each mL contains about 5 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, and sonicate for 20 minutes with occasional vigorous shaking. Centrifuge this solution at 4000 revolutions per minute for 15 minutes, filter the clear supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, then proceed as directed in the Assay.

$$\text{Amount [mg (potency)] of clarithromycin } (C_{38}H_{69}NO_{13}) = \text{Amount [mg (potency)] of Clarithromycin RS} \times \frac{Q_T}{Q_S} \times \frac{V}{100}$$

Internal standard solution (1)—A solution of butyl paraoxybenzoate in the mobile phase (1 in 1000)

Internal standard solution (2)—Pipet 1 mL of the internal standard solution (1), and add the mobile phase to make exactly 20 mL.

Dissolution Test Perform the test with 1 tablet of Clarithromycin Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution (pH 6.0) as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter

through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 28 μg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 28 mg (potency) of Clarithromycin RS, and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of clarithromycin in each solution. The dissolution rate of Clarithromycin Tablets in 30 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

W_S: Amount [mg (potency)] of Clarithromycin RS

C: Labeled amount [mg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$) in 1 tablet

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of clarithromycin is not more than 2.0.

System repeatability: When the test is repeated 6 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clarithromycin is not more than 2.0 %.

Assay To not less than 5 Clarithromycin Tablets add diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) so that each mL contains about 8 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, disperse to fine particles with the aid of ultrasonic waves, add 1 mL of the internal standard solution (1) per 100 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, then add acetonitrile for liquid chromatography so that each mL contains about 5 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) according to the labeled amount, and sonicate for 10 minutes with occasional vigorous shaking. Centrifuge this solution at 4000 revolutions per minute for 15 minutes, and filter the clear supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 20 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Clarithromycin RS, and dissolve in the mobile phase to make exactly 10 mL. Pipet 2 mL of this solution, add 2 mL of the internal standard solution (2) and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clarithromyicn to that of the internal standard.

Amount [μg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)

= Amount [μg (potency)] of Clarithromycin RS

$$\times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution (1)—A solution of butyl paraoxybenzoate in the mobile phase (1 in 1000)

Internal standard solution (2)—Pipet 1 mL of the internal standard solution (1), and add the mobile phase to make exactly 20 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50 °C

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 30) and acetonitrile for liquid chromatography (13 : 7)

Flow rate: Adjust the flow rate so that the retention time of clarithromycin is about 8 minutes.

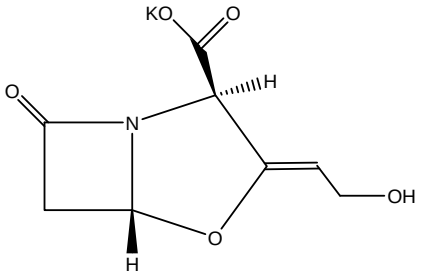
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Clavulanate Potassium



$C_8H_8KNO_5$: 237.25

Potassium(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*61177-45-5*]

Clavulanate Potassium contains not less than 810 μg (potency) and not more than 860 μg (potency) per mg of clavulanic acid ($C_8H_9NO_5$: 199.16), calculated on the anhydrous basis.

Description Clavulanate Potassium appears as white to pale yellowish white crystalline powder.
Clavulanate Potassium is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) To 1 mL each of solutions of Clavulanate Potassium and Clavulanic Acid RS (1 in 50000) add 5 mL of imidazole TS, warm in a water bath at 30 °C for 12 minutes, and allow to cool. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Clavulanate Potassium and Clavulanic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Clavulanate Potassium responds to the Qualitative Tests (1) for potassium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +53 ~ +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Clavulanate Potassium according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Clavulanate Potassium according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 0.1 g (potency) of Clavulanate Potassium, dissolve in the mobile phase A to make 10 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of the peak other than clavulanic acid from the test solution is not larger than the peak area of clavulanic acid from the standard solution. The total area of the peaks other than clavulanic acid from the test solution is not larger than 2 times the peak area of clavulanic acid from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 100 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihyrogen phosphate solution to 4.0 with phosphoric acid.

Mobile phase B: A mixture of mobile phase A and methanol (1 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-4	100	0
4-15	100→0	0→100
15-25	0	100

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of clavulanic acid obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from 20 μL of the standard solution.

System performance: Weigh accurately about 10 mg each of clavulanic acid and amoxicillin, and add the mobile phase A to make exactly 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of clavulnic acid and amoxicillin is not less than 8, and the number of theoretical plates of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clavulanic acid is not more than 2.0 %.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid

Water Not more than 1.5 % (5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Clavulanate Potassium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.03 EU/mg (potency) of clavulanate potassium, when Clavulanate Potassium is used in a sterile preparation.

Assay Weigh accurately about 12.5 mg (potency) each of Clavulanate Potassium and Clavulanic Acid RS, dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of clavulanic acid to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of clavulanic acid } (C_8H_9NO_5) = \text{Amount [μg (potency)] of Clavulanic Acid RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh accurately 0.3 g of sulfanilamide, dissolve in 30 mL of methanol, and add water to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 1.36 g of sodium acetate hydrate in 900 mL of water, adjust the pH to 4.5 with acetic acid (2 in 5), and add 30 mL of methanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of clavulanic acid is about 6 minutes.

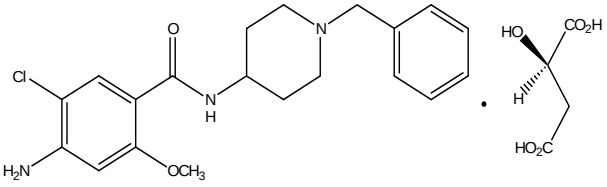
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, clavulanic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavulanic acid to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Clebopride Malate



and enantiomer

$C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$: 507.96

4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-benzylpiperidinium 3-carboxy-2-hydroxypropanoate [*57645-91-7*]

Clebopride Malate, when dried, containes not less than 98.5 % and not more than 101.0 % of clebopride malate ($C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$).

Description Clebopride Malate is a white, crystalline powder.

Clebopride Malate is sparingly soluble in methanol or in water, slightly soluble in ethanol (95) and practically insoluble in dichloromethane.

Melting point—About 164 °C (with decomposition).

Identification (1) Dissolve 20 mg of Clebopride Malate in 1 mL of surfuric acid, add 1 mL of 2-naphthol TS, mix and observe under daylight ; The solution shows a yellow color with blue fluorescence.

(2) Dissolve 20 mg each of Clebopride Malate and Clebopride Malate RS in water to make 100 mL, add water to 10 mL each of these solutions to make 100 mL and use these solutions as test solution and standard solution respactively. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Clebopride Malate and Clebopride Malate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Dissolve 5 mg of Clebopride Malate in ethanol (95) to make 10 mL and use this solution as the test solution. Separately, dissolve 5 mg of Clebopride Malate RS in ethanol (95) to make 10 mL and use this solution as the standard solution (1). Dissolve 5 mg of Clebopride Malate RS and 5 mg of metoclopramide hydrochloride RS in ethanol (95) to make 10 mL and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solutions (1) and (2) as bands of 10

mm on a plate of siliga gel with a fluorescent indicator for thin-layer chromatography. Develop the plates with a mixture of toluene, methanol, acetone and concentrated ammonia TS (70 : 14 : 14 : 2) to a distance of about 15 cm and air-dry the plate and examine under ultraviolet light (main wavelength : 254 nm). The main spot from test solution has the same R_f value as the spot from standard solution (1). This test is valid when the chromatogram obtained with standard solution (2) according to the above operation condition, shows two clearly separated bands.

2-naphthol solution—Dissolve 5g of 2-naphthol in 40 mL of 2 mol/L sodium hydroxide TS, add water to make 100 mL.

pH Dissolve 1.0 g of Clebopride Malate in water to make 100 mL: pH of this solution is between 3.8 and 4.2.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Clebopride Malate in water to make 100 mL: this solution is colorless and clear.

(2) ***Chloride***—Dissolve 1.0 g of Clebopride Malate in 20 mL of acetic acid (100), add 6 mL of dilute nitric acid, dilute with water to 50 mL and perform the test. Prepare the control solution, as follows mix 20 mL of acetic acid (100), 6 mL of nitric acid and 0.25 mL of 0.01 mol/L hydrochloric acid VS, and add water to make 50 mL (not more than 0.009 %).

(3) ***Sulphates***—Dissolve 2.0 g of Clebopride Malate in 20 mL of acetic acid (100), add water to make 50 mL and perform the test. Prepare the control solution, as follows mix 20 mL of acetic acid (100) and 0.42 mL of 0.005 mol/L sulfuric acid VS, and add water to make 50 mL (not more than 0.01 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Clebopride Malate according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Related substances***—Weigh accurately about 0.10 g of Clebopride Malate, dissolve in exactly 10 mL of the mobile phase, and use this solution as the test solution. Pipet 0.2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak other than clebopride from the test solution is not larger than the peak area of clebopride from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 3.85 g of ammonium acetate in water to make 500 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. To 400 mL of the filtrate add 600 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of clebopride is about 15 minutes.

System suitability

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of clebopride obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: Dissolve 30 mg of Clebopride Malate and 5 mg of propyl paraoxybenzoate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, propyl paraoxybenzoate and clebopride are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clebopride is not more than 2.5 %.

Time span of measurement: About 2 times as long as the retention time of clebopride.

Loss on Drying not more than 0.5 % (1 g, 105 °C, constant mass).

Residue on Ignition not more than 0.1 % (1 g).

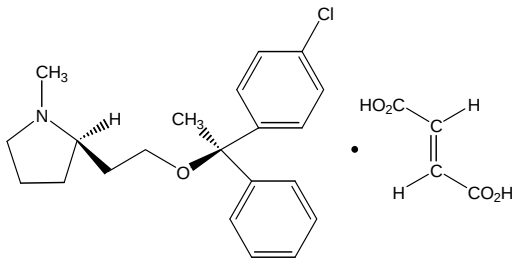
Assay Weigh accurately about 0.4 g of Clebopride Malate, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L Perchloric acid
= 50.80 mg of $C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Clemastine Fumarate



$C_{21}H_{26}ClNO \cdot C_4H_4O_4$: 459.96

(*E*)-but-2-Enedioic acid;(2*R*)-2-[2-[(1*R*)-1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine [*14976-57-9*]

Clemastine Fumarate, when dried, contains not less than 98.5 % and not more than 101.0 % of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$).

Description Clemastine Fumarate is a white, crystalline powder and is odorless.
Clemastine Fumarate is sparingly soluble in methanol or in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in ether and practically insoluble in water.

Identification (1) To 5 mg of Clemastine Fumarate, add 5 mL of sulfuric acid and shake to dissolve: a yellow color is observed. Slowly drop this solution into 10 mL of water: the yellow color immediately disappears.

(2) To 10 mg of Clemastine Fumarate, add 1 mL of fuming nitric acid and evaporate on a water-bath to dryness. Then add 2 mL of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc powder, heat for 10 minutes on a water-bath, cool and filter. Add 20 mL of water to the filtrate. The solution responds to the Qualitative Tests for primary aromatic amines.

(3) To 5 mL of a solution of Clemastine Fumarate (1 in 50000), add 5 mL of 4-dimethylaminobenzaldehyde TS and warm for 10 minutes: a red-purple color is observed.

(4) Perform the test with Clemastine Fumarate as directed under the Flame Coloration Test (2): a green color is observed.

(5) Dissolve 40 mg of Clemastine Fumarate and 10 mg of fumaric acid in 2 mL each of a mixture of ethanol (95) and water (4 : 1) by gentle warming and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, formic acid and water (90 : 7 : 3) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot with larger R_f value from the test solution has the same R_f value as the spot from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: +16 ~ +18° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Melting Point 176 ~ 180 ºC (with decomposition).

Purity (1) ***Clarity and color of solution*** —Dissolve 0.5 g of Clemastine Fumarate in 10 mL of methanol by warming: the solution is clear and colorless.

(2) ***Heavy metals***—Perform the test with 1.0 g of Clemastine Fumarate according to Method 2. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Clemastine Fumarate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related Substances***—Dissolve 0.10 g of Clemastine Fumarate in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 250 mL and use this solution as the standard solution (1). Pipet 5 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (90 : 10 : 1) to a distance of about 15 cm and air-dry the plate. After spraying evenly Dragendorff's TS on the plate, immediately spray evenly hydrogen peroxide TS: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (1) and not more than 2 spots from the test solution are more intense than the spot from the standard solution (2).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

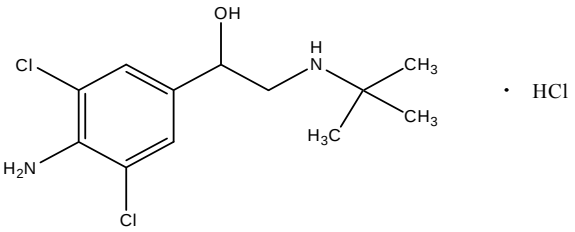
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.4 g of Clemastine Fumarate, previously dried, dissolved in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.00 mg of $C_{21}H_{26}ClNO \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Clenbuterol Hydrochloride



$C_{12}H_{18}Cl_2N_2O \cdot HCl$: 313.65

1-(4-Amino-3,5-dichlorophenyl)-2-(*tert*-butylamino)ethanol hydrochloride [*21898-19-1*]

Clenbuterol Hydrochloride contains not less than 99.0 % and not more than 101.0 % of clenbuterol hydrochloride ($C_{12}H_{18}Cl_2N_2O \cdot HCl$), calculated on the anhydrous basis.

Description Clenbuterol Hydrochloride is a white, crystalline powder.
Clenbuterol Hydrochloride is soluble in water or in ethanol (95) and slightly soluble in acetone.

Melting point—About 173 °C (with decomposition).

Identification (1) Determine the infrared spectra of Clenbuterol Hydrochloride and Clenbuterol Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 10 mg of Clenbuterol Hydrochloride in 10 mL of methanol and use this solution as the test solution. Separately, dissolve 10 mg of Clenbuterol Hydrochloride RS in 10 mL of methanol and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the with a plate mixture of toluene, ethanol (95) and ammonia solution (28) (15:10:0.15) a distance of about 10 cm, dry the plate. Splay evenly 1 % sodium nitrite solution in 1 mol/L hydrochloric acid, after standing for 10 minutes, dip the plate in 0.4 % naphthylethylenediamine dihydrochloride solution in methanol and remove the plate from the solution and air-dry the plate: the spots from the test solution and the standard solution show same color and same R_f value.

(3) A solution of Clenbuterol Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$**:** -0.10 ~ +0.10° (0.30 g, water, 10 mL, 100 mm)

pH Dissolve about 0.5 g of Clenbuterol Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***— Dissolve 0.5 g of Clenbuterol Hydrochloride in 10 mL of water: turbidity of this solution is not more intense than the following control suspension.

Control suspension—Dissolve 1.0 g of hydrazinium sufate in water to make exactly 100 mL, allow to stand for between 4 and 6 hours. Add 25.0 mL of this solution to the solution dissolving 2.5 g of hexamethyl tetraamine in water, mix well, allow to stand for 24 hours. Preserve in glass containers and use within 2 months. When this solution is used, add water to 15.0 mL of this suspension to make 1000 mL, mix well 90.0 mL of water and 10.0 mL of this suspension, and use this solution as the control suspension.

(2) ***Heavy metals***—Proceed with 1.0 g of Clenbuterol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.1 g of Clenbuterol Hydrochloride in the mobile phase to make exactly 50 mL, use this solution as the test solution. Dilute 0.1 mL of the test solution with water to make 100 mL, use this solution as the standard solution. Perform the test with 5μL each of test solution and the standard solution, as directed under Liquid Chromatography according to the following conditions, measure the peak area of each solution according to the automatic integration method. The area of any peak other than the principal peak from the test solution is not greater than the area of the principal peak from the standard solution (0.1 %) and the sum of the areas of all the peaks other than the principal peak form the test solution is not greater than 2 times the area of the principal peak from the standard solution (0.2 %). Disregard any peak with an area less than 0.1 times the area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet-visible absorption photometer (wavelength: 215 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).,

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of phosphate buffer solution, methanol and acetonitrile (60 : 20 : 20).

Flow rate : 0.5 mL/minute

System suitability

System performance: Dissolve 10 mg of Clenbuterol Related Substance I RS {1-(4-amino-3,5-dichloro-phenyl)-2-[(1,1-dimethylethyl)amino]ethanone (clenbuterol-ketone)}

with 20 mL of mobile phase, add 5 mL of the test solution, and add mobile phase to make 50 mL. When the procedure is run with 5μL of this solution as directed under the above operating conditions: the resolution between the related substance I peak and clebuterol peak is not less than 2.5.

Phosphate buffer solution—Dissolve sodium decanesulfonate and 5 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 3.0 with dilute phosphoric acid and dilute to 1000 mL with water.

Water Not more than 1.0 % (volumetric titration, direct titration).

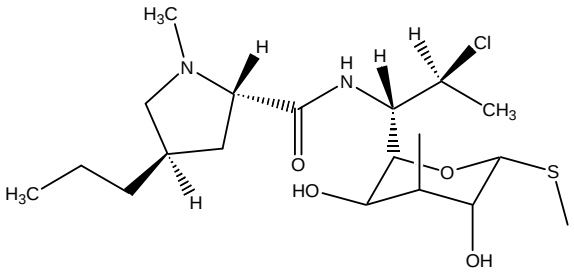
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Clenbuterol Hydrochloride in 50 mL of ethanol (95), add 5.0 mL of 0.01 mol/L hydrochloric acid, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration Endpoint Detection Method in Titrimetry). Read the volume added between 1st equivalent point and 2nd equivalent point of inflexion.

1 mL of 0.1 mol/L sodium hydroxide VS
= 31.365 mg of $C_{12}H_{18}Cl_2N_2O \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Clindamycin Hydrochloride



· HCl

$C_{18}H_{33}ClN_2O_5S \cdot HCl$: 461.44

(2*S*,4*R*)-*N*-{2-Chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(methylsulfanyl)oxan-2-yl]propyl}-1-methyl-4-propylpyrrolidine-2-carboxamide hydrochloride [*21462-39-5*]

Clindamycin Hydrochloride is the hydrochloride of a derivative of lincomycin.
Clindamycin Hydrochloride contains not less than 838 μg (potency) and not more than 940 μg (potency) per mg of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98), calculated on the anhydrous basis.

Description Clindamycin Hydrochloride appears as white to grayish white crystals or crystalline powder.
Clindamycin Hydrochloride is freely soluble in water or in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the infrared spectra of Clindamycin Hydrochloride and Clindamycin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Clindamycin Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +135 ~ +150° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1 g of Clindamycin Hydrochloride in 10 mL of water is between 3.0 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Clindamycin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately 0.125 g of Clindamycin Hydrochloride, add the mobile phase to make 25 mL, and use this solution as the test solution. Weigh accurately 50 mg of Lincomycin Hydrochloride RS and 100 mg of Clindamycin Hydrochloride RS, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine the peak areas of each solution by the automatic integration method, calculate the amount of lincomycin by equation (1), and calculate ethe amounts of 7-epiclindamycin, clindamycin B, and other related substances by equation (2): the amounts of 7-epiclindamycin, clindamycin b, and other related substances are not more than 4.0 %, not more than 2.0 %, and not more than 1.0 %, respectively, and the total amount of related substances including lincomycin is not more than 6.0 %.

Amount (%) of lincomycin ($C_{18}H_{34}N_2O_6S$)

$$= 2.5 \times \frac{C_L \times P_L}{W} \times \frac{A_T}{A_S} \quad (1)$$

C_L: Concentration (mg/mL) of lincomycin hydrochloride in the standard solution

P_L: Amount [μg/mg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$) in Lincomycin Hydrochloride RS

W: Amount (mg) of Clindamycin Hydrochloride taken

A_T: Peak area of lincomycin obtained from the test solution

A_S: Peak area of lincomycin obtained from the standard solution

$$\text{Amount (\%) of related substances} = 2.5 \times \frac{C \times P}{W} \times \frac{A_i}{A_C} \quad (2)$$

C: Concentration (mg/mL) of clindamycin hydrochloride in the standard solution

P: Amount [μg/mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in Clindamycin Hydrochloride RS

W: Amount (mg) of Clindamycin Hydrochloride taken

A_T: Peak area of each related substance obtained from the test solution

A_C: Peak area of clindamycin obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 5 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of a solution prepared by dissolving 6.8 g of potassium hydrogen phosphate in 1000 mL of water and adjusting the pH to 7.5 with 8 mol/L potassium hydroxide solution, and acetonitrile (550 : 450).

Flow rate: 1.0 mL/minute

System suitability

System performance: The relative retention times of lincomycin, clindamycin B, and 7-epiclindamycin with respect to clindamycin are 0.4, 0.65, and 0.9, respectively.

Time span of measurement: About 6 times as long as the retention time of clindamycin

Water Not more than 6.0 % (0.3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Clindamycin Hydrochloride is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Clindamycin Hydrochloride is used in a sterile preparation,. Weigh an appropriate amount of Clindamycin Hydrochloride, dissolve in Isotonic Sodium Chloride Injecton to make the solution so that each mL contains 5.0 mg, and use the solution as the test solution. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay Weigh accurately about 20 mg (potency) each of Clindamycin Hydrochloride and Clilndamycin Hydrochloride RS, dissolve in the mobile phase to make exactly 20 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas A_T and A_S, of clindamycin for the test solution and the standard solution.

$$\text{Amount [μg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = \text{Amount [μg (potency)] of Clindamycin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate adjusted to pH 7.5 with 8 mol/L potassium hydroxide solution, and acetonitrile (550 : 450)

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 10 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of clindamycin are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clindamycin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Clindamycin Hydrochloride Capsules

Clindamycin Hydrochloride Capsules contains not less than 93.0 % and not more than 107.0 % of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Mehtod of Preparation Prepare as directed under Capsules, with Clindamycin Hydrochloride.

Identification Weigh an amount of the contents of Clindamycin Hydrochloride Capsules, equivalent to 10

mg (potency) of clindamycin hydrochloride, add 2 mL of methanol, shake well, centrifuge, and use the clear supernatant liquid as the test solution. Separately, dissolve 10 mg of Clindamycin Hydrochloride RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, toluene, and ammonia solution (28) (140 : 60 : 3) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution prepared by adding 50 mL of bismuth subnitrate to 500 mL of a solution of L-tartaric acid (1 in 5) on the plate: the principal spot obtained from the test solution shows the same R_f value as the spot from the standard solution.

Water Not more than 7.0 % (0.2 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 capsule of Clindamycin Hydrochloride Capsules at 50 revolutions per minute according to Method 2, using 900 mL of water as the dissolution solution. Take not less thn 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 83 μg (potency) of clindamycin hydrochloride ($C_{18}H_{33}ClN_2O_5S$), and use this solution as the test solution. Separately, weigh accurately about 17 mg (potency) of Clindamycin Hydrochloride RS, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of clindamycin in each solution. The dissolution rate of Clindamycin Hydrochloride Capsules in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of clindamycin hydrochloride ($C_{18}H_{33}ClN_2O_5S$)
= Amount [mg (potency)] of Clindamycin Hydrochloride RS $\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 450$

C: Labeled amount [mg (potency)] of clindamycin hydrochloride ($C_{18}H_{33}ClN_2O_5S$) in 1 capsule

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of 0.05 mol/L potassium hydrogen phosphate TS adjusted to pH 7.5 with 8 mol/L potassium hydroxide TS, and acetonitrile (550 : 450)

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 7 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clindamycin hydrochloride is not more than 2.0 %.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clindamycin Hydrochloride Capsules add the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly V mL so that each mL contains 0.75 mg (potency) of clindamycin hydrochloride according to the labeled amount. Centrifuge this solution, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 75 mg (potency) of Clindamycin Hydrochloride RS, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount [μg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$)
= Amount [μg (potency)] of Clindamycin Hydrochloride RS $\times \frac{A_T}{A_S} \times \frac{V}{100}$

Assay Weigh accurately the mass of contents of not less than 20 Clindamycin Hydrochloride Capsules. Weigh accurately an amount of the contents, equivalent to about 75 mg (potency) of clindamycin according to the labeled potency, add the mobile phase, shake for 30 minutes, make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 75 mg (potency) of Clindamycin Hydrochloride RS, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of clindamycin in each solution.

Amount [μg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$)
= Amount [μg (potency)] of Clindamycin Hydrochloride RS $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of 8 mol/L potassium hydroxide solution adjusted to pH 7.5 with 0.05 mol/L potassium dihydrogen phosphate, and acetonitrile (550 : 450)

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 7 minutes.

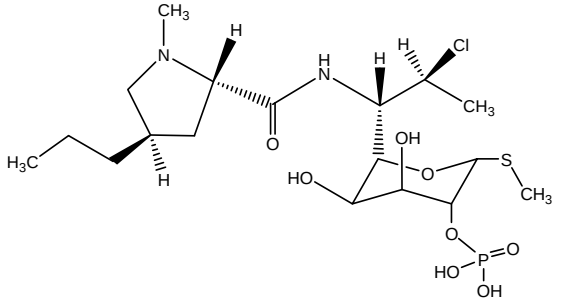
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of clindamycin is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clindamycin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Clindamycin Phosphate



$C_{18}H_{34}ClN_2O_8PS$: 504.97

Methyl 5-{2-chloro-1-[(1-methyl-4-propylprolyl)amino]propyl}-2-O-phosphono-1-thiopentopyranoside [*24729-96-2*]

Clindamycin Phosphate is a derivative of clindamycin. Clindamycin Phosphate contains not less than 800 μg (potency) and not more than 846 μg (potency) per mg of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98), calculated on the anhydrous basis.

Description Clindamycin Phosphate is a whtie to pale yellowish white crystalline powder.
Clindamycin Phosphate is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification Determine the infrared spectra of Clindamycin Phosphate and Clindamycin Phosphate, previously dried at 100 °C for 2 hours, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +115 ~ +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Clindamycin Phosphate in 10 mL of water is between 3.5 and 4.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Clindamycin Phosphate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Clindamycin Phosphate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Clindamycin Phosphate according to Method 4, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately 0.1 g of Clindamycin Phosphate, dissolve in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exadtly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the peak area of clindamycin having the relative retention time of about 1.8 with respect to clindamycin phosphate from the test solution is not larger than 1/2 times the peak area of clindamycin phsosphate from the standard solution. The total area of the peaks other than clindamycin phosphate from the test solution is not larger than 4 times the peak area of clindamycin phosphate from the standard solution.

Internal standard solution—A solution of methyl paraoxybenzoate in the mobile phase (3 in 50000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of clindamycin phosphate is about 8 minutes.

System suitability

Test for required detectability: Pipet 1.0 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin phosphate obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: Weigh accurately an amount of Clindamycin Phosphate RS, equivalent to 20 mg (potency), in 25 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5 %.

Time span of measurement: About 2 times as long as the retention time of clindamycin phosphate, beginning after the solvent peak

Water Not more than 6.0 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Clindamycin Phosphate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.58 EU/mg (potency) of clindamycin phosphate, when Clindamycin Phosphate is used in a sterile preparation.

Histamine It meets the requirement, when Clindamycin Phosphate is used in a sterile preparation. Weigh appropriate an amount of Clindamycin Phosphate, dissolve in Isotonic Sodium Chloride Injection, make a solution so that each mL contains 5 mg (potency), and use the solution as the test solution.

Assay Weigh accurately an amount each of Clindamycin Phosphate and Clindamycin Phosphate RS, equivalent to about 20 mg (potency), dissolve each in exactly 25 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = \text{Amount [μg (potency)] of Clindamycin Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl paraoxybenzoate in the mobile phase (3 in 50000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, and adjust the pH to 2.5 with phosphoric acid. To this solution add 225 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin phosphate is about 8 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5 %.

Containers and Storage ***Containers***—Tight containers.

Clindamycin Phosphate Gel

Clindamycin Phosphate Gel contains not less than 90.0 % and not more than 120.0 % of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.99).

Method of Preparation Prepare as directed under Gels, with Clindamycin Phosphate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH 5.5 ~ 7.5.

Assay Weigh accurately an amount of Clindamycin Phosphate Gel, equivalent to about 20 mg (potency), add exactly 20.0 mL of the internal standard solution, dissolve completely, add the mobile phase to make exactly 100 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg of Clindamycin Phosphate RS, add exactly 20.0 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = \text{Amount [μg (potency)] of Clindamycin Phosphate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve about 60 mg of methyl paraoxybenzoate in the mobile phase to make exactly 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: About 1.0 mL/minute

System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clindamycin is not more than 2.5 %.

Containers and Storage ***Containers***—Tight containers.

Clindamycin Phosphate Injection

Clindamycin Phosphate Injection is an aqueous solution for injection.

Clindamycin Phosphate Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$: 504.96).

Method of Preparation Prepare as directed under Injections, with Clindamycin Phosphate.

Description Clindamycin Phosphate Injection appears as a clear, colorless to pale yellow liquid.

Identification To a volume of Clindamycin Phosphate Injection, equivalent to 0.15 g (potency) of clindamycin phosphate according to the labeled amount, add 4 mL of water, 2 mL of 8 mol/L sodium hydroxide TS, and 0.1 mL of sodium pentacyanonitrosylferrate (III) TS, shake, heat in a water bath for 10 minutes, and add 2 mL of hydrochloric acid: a blue-green color develops.

pH 6.0 ~ 7.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.1 EU/mg (potency) of clindamycin phosphate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Proceed as directed in the Assay under Clindamycin Phosphate. Weigh accurately an amount of Clindamycin Phosphate Injection, equivalent to about 0.3 g (potency) according to the labeled potency, and add the mobile phase to make exactly 100 mL. Pipet 7 mL of this solution, add exactly 25 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Clindamycin Phosphate RS, dissolve in exactly 25 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution.

$$\text{Amount [mg (potency)] of clindamycin phosphate } (C_{18}H_{34}ClN_2O_8PS) = \text{Amount [mg (potency)] of Clindamycin Phosphate RS} \times \frac{Q_T}{Q_S} \times \frac{100}{7}$$

Internal standard solution—A solution of methyl paraoxybenzoate in the mobile phase (3 in 50000)

Containers and Storage ***Containers***—Hermetic containers.

Clindamycin Phosphate Topical Solution

Clindamycin Phosphate Solution

Clindamycin Phosphate Topical solution is a topical solution.
Clindamycin Phosphate Topical Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.99).

Method of Preparation Prepare as directed under Solutions, with Clindamycin Phosphate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH 4.0 ~ 7.0.

Assay Pipet a volume of Clindamycin Phosphate Topical Solution, equivalent to about 20 mg (potency) according to the labeled potency, add exactl 25.0 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Clinadmycin Phosphate RS, dissolve in exactly 25.0 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard in the test solution and standard solution.

$$\text{Amount [μg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = \text{Amount [μg (potency)] of Clindamycin Phosphate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve about 40 mg of 4-hydroxyacetophenone in 10 mL of acetonitrile, and add the mobile phase so that each mL contains 0.04 mg.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: About 1.0 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of clindamycin and 4-hydroxyacetophenone is not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin to that of the internal standard is not more than 2.5 %.

Containers and Storage ***Containers***—Tight containers.

Clindamycin Phosphate Vaginal Cream

Clindamycin Phosphate Vaginal Cream contains not less than 90.0 % and not more than 120.0 % of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.99).

Method of Preparation Prepare as directed under Creams, with Clindamycin Phosphate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH 3.0 ~ 6.0.

Assay Weigh accurately an amount of Clindamycin Phosphate Vaginal Cream, equivalent to about 20 mg (potency), add exactly 20.0 mL of the internal standard solution, dissolve completely, add the mobile phase to make exactly 100 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg (potency) of Clindamycin Phosphate RS, add exactly 20.0 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard.

$$\text{Amount [μg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = \text{Amount [μg (potency)] of Clindamycin Phosphate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve about 60 mg of methyl paraoxybenzoate in the mobile phase to make exactly 1 L.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 250 mm in length, packed with octylsilyl silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

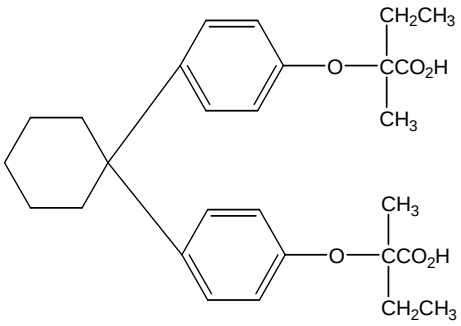
Flow rate: About 1.0 mL/minute

System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clindamycin is not more than 2.5 %.

Containers and Storage ***Containers***—Tight containers.

Clinofibrate



$C_{28}H_{36}O_6$: 468.58

2-[4-[1-[4-(2-Carboxybutan-2-yloxy)phenyl] cyclohexyl]phenoxy]-2-methylbutanoic acid [*30299-08-2*]

Clinofibrate, when dried, contains not less than 98.5 % and not more than 101.0 % of clinofibrate ($C_{28}H_{36}O_6$).

Description Clinofibrate is a white to yellowish white powder, is odorless and has no taste.

Clinofibrate is freely soluble in methanol, in ethanol (99.5), in acetone or in ether and practically insoluble in water.

A solution of Clinofibrate in methanol (1 in 20) shows no optical rotation.

Melting point—About 146 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Clinofibrate and Clinofibrate RS in ethanol (99.5) (1 in 50000) under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Clinofibrate and Clinofibrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Clinofibrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Clinofibrate according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Clinofibrate in 10 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution and add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 50 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane and acetic acid (100) (12 : 5 : 3) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Isomer ratio To 50 mg of Clinofibrate, add 0.4 mL of thionyl chloride, stopper tightly, heat in a water-bath at 60 °C for 5 minutes with occasional shaking and evaporate the excess thionyl chloride at a temperature not exceeding 60 °C under reduced pressure. Dissolve the residue in 2 mL of toluene, previously dried with synthetic zeolite for drying, add 2 mL of a solution of D-(+)-α-methyl-benzylamine in toluene, previously dried with synthetic zeolite for drying (3 in 100), mix gently, allow to stand for 10 minutes and evaporate the toluene at a temperature not exceeding 60 °C under reduced pressure. Dissolve the residue in 5 mL of chloroform and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area, A_a, A_b and A_c, of three peaks appear in order near the retention time of 40 minutes: a value, $A_b/(A_a+A_b+A_c)\times 100$, is between 40 and 70.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20 °C.

Mobile phase: A mixture of hexane and 2-propanol (500 : 3).

Flow rate: Adjust the flow rate so that the retention time of the peak appearing first is about 35 minutes.

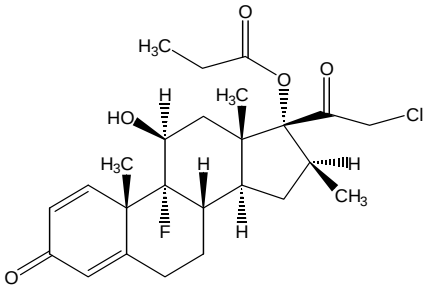
Selection of column: Proceed with 5 μL of the test solution under the above operating conditions. Use a column giving a complete separation of the three peaks.

Assay Weigh accurately about 0.45 g of Clinofibrate, previously dried, dissolve in 40 mL of ethanol (99.5), add 30 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.429 mg of $C_{28}H_{36}O_6$

Containers and Storage ***Containers***—Tight containers.

Clobetasol Propionate



$C_{25}H_{32}ClFO_5$: 466.97

[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-17-(2-Chloroacetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[*a*]phenanthren-17-yl]propanoate [*25122-46-7*]

Clobetasol Propionate, when dried, contains not less than 97.0 % and not more than 102.0 % of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

Description Clobetasol Propionate appears as white to pale yellow crystalline powder.

Clobetasol Propionate is soluble in methanol or in ethanol (95), and practically insoluble in water.

Clobetasol Propionate is gradually colored to yellow by light.

Melting point—196 °C (with decomposition).

Identification (1) Determine the infrared spectra of Clobetasol Propionate and Clobetasol Propionate RS, previously dried, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation, both relative to the internal standard, as obtained in the Assay.

Specific Optical Rotation $[\alpha]_D^{20}$: +112 ~ +118° (0.5 g after drying, acetone, 50 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Clobetasol Propionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh 10.0 mg of Clobetasol Propionate, dissolve in the mobile phase to make 100 mL and use this solution as the test solution (1). Pipet 1 mL of the test solution (1), add the mobile phase to make exactly 50 mL and use this solution as the test solution (2). Separately, weigh accurately about 10 mg of Clobetasol Propionate RS and about 5 mg of 9-Fluoro-11-hydroxy-16-methyl-3-oxoandrosta-1,4-diene-17(*R*)-spiro-2'-[4'-chloro-5'-ethylfurane-3-(2'*H*)-one] RS, dissolve in the mobile phase to make 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solutions (1) and (2) and the standard solution as directed under Liquid Chromatography: the area of each peak other than the principal peak of the test solution (1) is not more than 0.5 times (1.0 %) of the area of the principal peak of the test solution (2) and the total peak areas of all related substances is not more than 1.25 times (2.5 %) of the area of the principal peak of the test solution (2). But, omit the peak which peak area is less than 0.025 times (0.05 %) of the area of the principal peak among the peaks of the test solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture acetonitrile, 0.05 mol/L sodium dihydrogen phosphate solution (adjust to pH 2.5 with phosphoric acid, and methanol (475 : 425 : 100).

Flow rate: 1 mL/minute.

System suitability

Selection of column: Proceed with 10 μL of the

standard solution according to the above operating conditions. Use a column wih the resolution between the peaks of clobetasol propionate and 9α-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17(*R*)-spiro-2'-[4'-chloro
-5'-ethylfurane-3-(2'*H*)-one] being not less than 3.0.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 10 mg each of Clobetasol Propionate and Clobetasol Propionate RS, previously dried, dissolve each in mobile phase, add exactly 100 mL of the internal standard solution, add mobile phase to make 250 mL and use these solution as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions of the related substances in Purity and calculate the ratios, Q_T and Q_S, of the peak area of clobetasol propionate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of clobetasol propionate } (C_{25}H_{32}ClFO_5) = \text{Amount (mg) of Clobetasol Propionate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of Beclometasone Dipropionate in mobile phase (1 in 5000).

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Clobetasol Propionate Cream

Clobetasol Propionate Cream contains not less than 90.0 % and not more than 115.0 % of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$:466.97).

Method of Preparation Prepare as directed under Creams, with Clobetasol Propionate.

Identification (1) Transfer, accurately weighed, a portion of Clobetasol Propionate Cream, equivalent to about 0.75 mg of clobetasol propionate ($C_{25}H_{32}ClFO_5$), to a 25 mL centrifugal tube, add 10 mL of methanol, stopper, heat in the water-bath at 60 °C for 4 minutes, then take out from the water-bath and extract while vigorously shaking. Repeat this procedure several times, cool to room temperature, add 3.5 mL of water and centrifuge for about 10 minutes. Transfer 10 mL of the clear supernatant liquid to a separator, add 1 g of sodium chloride and 10 mL of water and mix well. To this solution, add 5 mL of dichloromethane, extract after sonication for 1 minute, take the dichloromethane layer, evaporate to dryness under the current of nitrogen, dissolve the residue in 0.5 mL of dichloromethane and use this solution as the test solution. Proceed as directed in the Identification (1) under Clobetasol Propionate Ointment.

(2) The retention time of the principal peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation, both relative to the internal standard, as obtained in the Assay.

Assay Weigh accurately a portion of Clobetasol Propionate Cream, equivalent to about 1 mg of clobetasol propionate ($C_{25}H_{32}ClFO_5$), add 10 mL of ethanol (99.5), warm in a water-bath with frequent shaking until the cream is completely dispersed and cool in the ice-bath for 30 minutes. Pipet 5 mL of the clear supernatant liquid after centrifuge, add 5.0 mL of the internal standard solution, mix well and use this solution as the test solution. With this test solution, proceed as directed in the Assay under Clobetasol Propionate Ointment.

$$\text{Amount (mg) of clobetasol propionate}(C_{25}H_{32}ClFO_5) = \text{Amount (mg) of Clobetasol Propionate RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of Beclometasone Dipropionate in 50 % ethanol (1 in 5000).

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Clobetasol Propionate Ointment

Clobetasol Propionate Ointment contains not less than 90.0 % and not more than 115.0 % of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$: 466.97).

Method of Preparation Prepare as directed under Ointments, with Clobetasol Propionate.

Identification (1) Transfer a portion of Clobetasol Propionate Ointment, equivalent to about 0.5 mg of clobetasol propionate ($C_{25}H_{32}ClFO_5$) according to the labled amount, to a 25 mL centrifugal tube, add 10 mL of methanol, stopper, heat in the water-bath at 70 °C for 4 minutes, then take out from water-bath and extract while vigorously shaking. Repeat this operation several times, cool in the ice-bath for 5 minutes and centrifuge for about 10 minutes. Pipet 5 mL of the clear supernatant liquid, evaporate to dryness under the current of nitrogen, dissolve the residue in 0.5 mL of di-

chloromethane and use this solution as the test solution. Separately, weigh about 10 mg of Clobetasol Propionate RS, dissolve in dichloromethane to make 20 mL and use this solution as the standard solution. Perform the test with these solutions as directed and the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethanes, acetone and ethanol (99.5) (100 : 10 : 5) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 245 nm): the principal spot from the test solution shows the same R_f value as the principal spot from the standard solution.

(2) The retention time of the principal peak in the chromatogram of the test solution in Assay corresponds to that of the standard solution, both relative to the internal standard, as obtained in the Assay.

Assay Weigh accurately a portion of Clobetasol Propionate Ointment, equivalent to about 1 mg of clobetasol propionate ($C_{25}H_{32}ClFO_5$), add 10 mL of ethanol (99.5), warm in the water-bath with frequent shaking until the ointment is completely dispersed and cool in the ice-bath for 30 minutes. Pipet 5 mL of the clear supernatant liquid after centrifuge, add 5 mL of the internal standard solution, mix well and use this solution as the test solution. Separately, weigh accurately about 10 mg of Clobetasol Propionate RS, dissolve in 50 % ethanol to make 100 mL, transfer exactly 5 mL of this solution to a volumetric flask, add 5.0 mL of the internal standard solution, mix well and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according operating to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Clobetasol Propionate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of clobetasol propionate}(C_{25}H_{32}ClFO_5) = \text{Amount (mg) of Clobetasol Propionate RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of Beclometasone Dipropionate in 50 % ethanol (1 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 10 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and ethanol (99.5) (55 : 45).

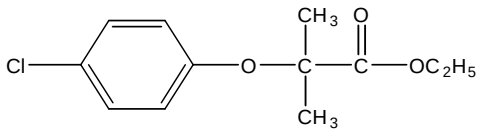
Column temperature: 60 °C.

Flow rate: 2 mL/minute.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Clofibrate



$C_{12}H_{15}ClO_3$: 242.70

Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate
[*637-07-0*]

Clofibrate contains not less than 98.0 % and not more than 101.0 % of clofibrate ($C_{12}H_{15}ClO_3$), calculated on the anhydrous basis.

Description Clofibrate is a colorless or pale yellow, clear, oily liquid, has a characteristic odor and taste, which is bitter at first and subsequently sweet.
Clofibrate is miscible with methanol, with ethanol (95), with ethanol (99.5), with ether or with hexane and practically insoluble in water.
Clofibrate is gradually decomposed by light.

Identification (1) Determine the absorption spectra of solutions of Clofibrate and Clofibrate RS in ethanol (99.5) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similsr intensities of absorption at the same wavelengths. Determine the absorption spectra of solutions of Clofibrate and Clofibrate RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Clofibrate and Clofibrate RS as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20} : 1.500 ~ 1.505.

Specific Gravity d_{20}^{20} : 1.137 ~ 1.144.

Purity (1) ***Acid***—Dissolve 2.0 g of Clofibrate in 100 mL of neutralized ethanol and add 1 drop of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) ***Heavy metals***—Proceed with 2.0 g of Clofibrate according to Method 2 and perform the test.

Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—To 5.0 g of Clofibrate, add 20 mL of nitric acid and 5 mL of sulfuric acid and heat until white fumes are evolved. After cooling, if necessary, add further 5 mL of nitric acid, heat until white fumes are evolved and repeat this procedure until the solution is colorless to pale yellow. After cooling, add 15 mL of saturated ammonium oxalate solution and heat again until white fumes are evolved. Cool, add water to make 25 mL, use 5 mL of this solution as the test solution and perform the test (not more than 2 ppm).

Standard solution—Prepare a solution according to the above procedure without using Clofibrate as the blank. Transfer 5 mL of the solution to a generator bottle, add 2.0 mL of standard arsenic solution and then proceed as directed in the test solution.

(4) ***4-Chlorophenol and other related substances***—Pipet 10.0 mL of Clofibrate, add 5.0 μL of tributyrin, and use this solution as the test solution. Separately, weigh accurately 50 mg of Clofibrate RS and 15 mg of 4-chlorophenol, dissolve in the chloroform to make 50 mL, pipet 1 ml of this solution, and add chloroform to make 10 mL so that each mL contains 0.1 mg of clofibrate and 0.03 mg of 4-chlorophenol. Pipet 10.0 mL of this solution, add 5.0 μL of tributyrin, and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratio, Q_i, of the peak area of each related substance other than 4-chlorophenol to that of tributyrin obtained from the test solution, and the ratio, Q_S, of the peak area of clofibrate to that of tributyrin from the standard solution, by equation (1): the amount of each related substance other than 4-chlorophenol is not more than 0.01 %, and the total amount of related substances is not more than 0.12 %. Calculate the ratio, Q_{Tc}, of the peak area of 4-chlorophenol to that of tributyrin from the test solution, and the ratio, Q_{Sc}, of the peak area of 4-chlorophenol to that of tributyrin from the standard solution, by equation (2): the amount of 4-chlorophenol is not more than 0.003 %.

$$\text{Amount (\%) of related substances} = 0.1 \times C \times \frac{Q_i}{Q_S} \quad (1)$$

C: Concentration (mg/mL) of clofibrate in the standard solution

$$\text{Amount (\%) of } p\text{-chlorophenol } (ClC_6H_4OH) = 0.1 \times C \times \frac{Q_{Tc}}{Q_{Ss}} \quad (2)$$

C: Concentration (mg/mL) of 4-chlorophenol in the standard solution

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 0.53 mm in internal diameter and about 15 m in length, the inside coated with dimethylpolysiloxane oil 1.5 μm in thickness.

Column temperature: Maintain at 120 °C for the first 1 minute, raise the temperature to 180 °C at the rate of 5 °C per minute for the next 12 minutes, and maintain at 180 °C for 9 minutes.

Carrier gas: Helium

Flow rate: 2 mL/minute

Injection port temperature: 210 °C

Detector temperature: 220 °C

Split ratio: About 1 : 20

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the relative retention times of 4-chlorophenol and clofibrate with respect to tributyrin are about 0.2 and about 0.55, respectively.

Water Not more than 0.2 % (5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Clofibrate, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol VS and heat in a water-bath under a reflux condenser with a carbon dioxide absorbing tube (soda lime) for 2 hours with frequent shaking. Cool and titrate immediately the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary connection.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.270 mg of $C_{12}H_{15}ClO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Clofibrate Capsules

Clofibrate Capsules contain not less than 93.0 % and not more than 107.0 % of the labeled amount of clofibrate ($C_{12}H_{15}C1O_3$: 242.70).

Method of Preparation Prepare as directed under Capsules, with Clofibrate.

Identification Open the capsules and use the contents. Determine the absorption spectrum of a solution of the contents in ethanol (99.5) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 278 nm and 282 nm. Determine the absorption spectrum of a solution of the

contents in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 224 nm and 228 nm.

Purity ***4-Chlorophenol***—Cut and open not less than 20 Clofibrate Capsules and proceed with 1.0 g of the well mixed contents as directed in the Purity (4) under Clofibrate.

Dissolution Test Perform the test with 1 capsule of Clofibrate Capsules at 100 revolutions per minute according to Method 2, using 1000 mL of a solution of sodium lauryl sulfate (5 in 100) as the dissolution solution. Pipet 5.0 mL of the dissolved solution 180 minutes after the start of the test, add methanol to make exactly 25.0 mL, allow to stand for 5 minutes, filter, and use the filtrate as the test solution. Separately, weigh accurately 20 mg of Clofibrate RS, add 20 mL of methanol, mix well to dissolve, and add water to make 50 mL. To a suitable amount of this solution add methanol so that each mL contains about 80 μg, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of the peak area of clofibrate in each solution. The dissolution rate of Clofibrate Capsules in 180 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of clofibrate ($C_{12}H_{15}ClO_3$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 500000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of clofibrate ($C_{12}H_{15}ClO_3$) in 1 capsule

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 226 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: A mixture of methanol and water (80 : 20)

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of clofibrate is not more than 1.5.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clofibrate is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement

Assay Weigh accurately not less than 20 Clofibrate Capsules, open the capsules, rinse the inside of the capsules with a small amount of ether after taking out the contents, evaporate the ether by allowing the capsules to stand at room temperature and weigh the capsules accurately. Weigh accurately an amount of the contents, equivalent to about 0.1 g of clofibrate ($C_{12}H_{15}C1O_3$), dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Clofibrate RS, proceed in the same manner as directed for the test solution and use this solution so obtained as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Clofibrate to that of the internal standard, for the test solution and the standard solution, respectively.

Amount (mg) of cloabrate ($C_{12}H_{15}C1O_3$)
= Amount (mg) of Clofibrate RS,
calculated on the anhydrous basis $\times \frac{Q_T}{Q_S}$

Interanl standard solution—A solution of ibuprofen in the mobile phase (1 in 100).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3 : 2).

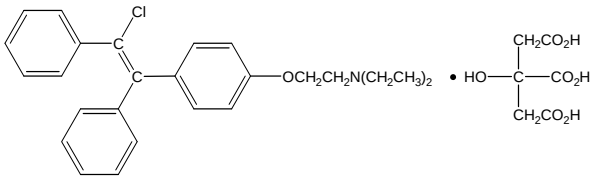
Flow rate: Adjust the flow rate so that the retention time of Clofibrate is about 10 minutes.

Selection of column: Dissolve 50 mg of Clofibrate and 0.3 g of ibuprofen in 50 mL of acetonitrile. Proceed with 10 μL of this solution under the above operating conditions and calculate the resolution. Use a column giving elution of ibuprofen and clofibrate in this order with the resolution between their peaks being not less than 6.0.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Clomifene Citrate



$C_{26}H_{28}ClNO \cdot C_6H_8O_7$: 598.08

2-[4-[(*Z*)-2-Chloro-1,2-diphenylethenyl]phenoxy]-*N,N*-diethylethanamine;2-hydroxypropane-1,2,3-tricarboxylic acid [*50-41-9*]

Clomifene Citrate, when dried, contains not less than 98.0 % and not more than 101.0 % of clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$).

Description Clomifene Citrate is a white to pale yellowish white powder and is odorless.
Clomifene Citrate is freely soluble in methanol or in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in ether.
Clomifene Citrate is gradually colored by light.

Melting point—About 115 °C

Identification (1) To 2 mL of a solution of Clomifene Citrate in butanol (1 in 200), add 2 mL of Reinecke salt TS: a pale red precipitate is produced.

(2) Determine the absorption spectra of solutions of Clomifene Citrate and Clomifene Citrate RS in 0.1 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Clomifene Citrate in methanol (1 in 200) responds to the Qualitative Tests (1) and (2) for citrate salt.

Purity (1) ***Clarity and color of solution***—The solution of 1.0 g of Clomifene Citrate in 30 mL of methanol is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Clomifene Citrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Weigh accurately 50 mg of Clomifene Citrate, and add the mobile phase to make 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately 50 mg of Clomifene Citrate RS, and dissolve in the mobile phase to make 100 mL. Pipet 1 mL of this solution, add the mobile phase to make 50 mL, pipet 1 mL of this solution, add the mobile phase to make 10 mL so that each mL contains about 1.0 μg, and use this solution as the standard solution. Perform the test with 50 μL of the test solution as directed in the area percentage method under Liquid Chromatography according to the following conditions: the amount of clomifene related substance I {(*E,Z*)-2-[4-(1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine hydrochloride} obtained from the test solution is not more than 2.0 %, the amount of each related substance is not more than 0.5 %, and the total amount of related substances is not more than 2.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 290 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with butylsilyl silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phae: Adjust the pH of a mixture of methanol, water, and triethylamine (55 : 45 : 0.3) to 2.5 with phosphoric acid.

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately a suitable amount each of Clomifene Related Substance I RS and Clomifene Citrate RS, make a solution so that each mL contains 0.002 mg of clomifene related substance I and 0.05 mg of clomifene citrate, and use this solution as the system suitability solution. When the procedure is run with 50 μL of this solution under the above operating conditions, the relative retention times of clomifene related substance I, (*Z*)-isomer, and (*E*)-isomer are about 0.9, about 1.0, and about 1.2, respectively, and the resolutions between the peaks of clomifene related substance I and (*Z*)-isomer and between the peaks of (*Z*)-isomer and (*E*)-isomer are not less than 1.0 and not less than 1.5, respectively. When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of (*E*)-isomer are not less than 2000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 5 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (*E*)-isomer and (*Z*)-isomer is not more than 2.0 %.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, P_2O_5, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Isomer Ratio To 0.10 g of Clomifene Citrate, add 10 mL of water and 1 mL of sodium hydroxide TS and extract with three 15 mL volumes of ether. Wash the combined ether extracts with 20 mL of water, add 10 g of anhydrous sodium sulfate to the combined ether extracts, shake for 1 minute, filter and evaporate the ether of the filtrate. Dissolve the residue in 10 mL of chloroform and use this solution as the test solution. Perform the test with 2 μL of the test solution as di-

rected under Gas Chromatography according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having retention times of about 20 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a+A_b)$ is between 0.3 and 0.5.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and about 1 m in length, packed with siliceous earth for gas chromatography (125 μm to 150 μm in particle diameter), coated with methylsilicone polymer for gas chromatography at the ratio of 1 %.

Column temperature: A constant temperature of about 195 °C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the peak for the first elution of clomifene citrate is about 20 minutes.

System suitability

Selection of column: Proceed with 2 μL of the test solution under the above operating conditions: use a column giving the resolution between the two peaks of clomifene citrate being not less than 1.3.

Assay Weigh accurately about 1 g of Clomifene Citrate, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.81 mg of $C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Clomifene Citrate Tablets

Clomifene Citrate Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of the clomifene citrate ($C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$: 598.08).

Method of Preparation Prepare as directed under Tablets, with Clomifene Citrate.

Identification (1) Weigh a portion of powdered Clomifene Citrate Tablets, equivalent to 1 g of Clomifene Citrate according to the labeled amount, shake vigorously with 100 mL of chloroform and filter. Concentrate the filtrate in a water-bath, allow to stand at room temperature, collect the crystals formed and wash with a small volume of chloroform. Proceed with the crystals as directed in the Identification (1) and (3) under Clomifene Citrate.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) in 0.1 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 233 and 237 nm and between 290 and 294 nm.

Dissolution Test Perform the test with 1 tablet of Clomifene Citrate Tablets at 100 revolutions per minute according to Method 1, using 900 mL of water as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Clomifene Citrate RS, dissolve in water to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 232 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Clomifene Citrate Tablets in 30 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of clomifene citrate ($C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$) in 1 tablet

Uniformity of Dosage Limits It meets the requirement

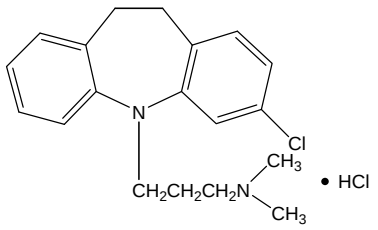
Assay Weigh accurately and powder not less than 20 Clomifene Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of clomifene citrate ($C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$), add 50 mL of methanol, shake for 10 minutes and add methanol to make exactly 100 mL. Centrifuge a volume of this solution, pipet 4.0 mL of the clear supernatant liquid, add methanol to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately 50 mg of Clomifene Citrate RS, previously dried in a desiccator (in vacuum, P_2O_5) for 3 hours and dissolve in methanol to make 100 mL. Pipet 4.0 mL of this solution and dilute with methanol to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry, A_T and A_S, of the test solution and the standard solution, respectively, at 295 nm.

Amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO{\cdot}C_6H_8O_7$)

$$= \text{Amount (mg) of Clomifene Citrate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Clomipramine Hydrochloride



$C_{19}H_{23}ClN_2 \cdot HCl$: 351.31

(3-{5-Chloro-2-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3(8),4,6,12,14-hexaen-2-yl}propyl) dimethylamine hydrochloride [*17321-77-6*]

Clomipramine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$).

Description Clomipramine Hydrochloride is a white to pale yellow, crystalline powder and is odorless.
Clomipramine Hydrochloride is very soluble in acetic acid (100), freely soluble in water, in methanol or in chloroform, soluble in ethanol (95), sparingly soluble in acetic anhydride, slightly soluble in acetone and practically insoluble in ethyl acetate or in ether.

Identification (1) Dissolve 3 mg of Clomipramine Hydrochloride in 1 mL of nitric acid: a deep blue color is observed.

(2) Determine the absorption spectra of solutions of Clomipramine Hydrochloride and Clomipramine Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Take 1 g of Clomipramine Hydrochloride in a separator, dissolve in 10 mL of water, add 5 mL of sodium hydroxide TS and extract with two 30 mL volumes of ether [the water layer is used for the Identification (4)]. Combine the ether extracts, add 20 mL of water and shake. Take ether layer, dry with a small portion of anhydrous sodium sulfate and filter. Evaporate the combined extracts by warming a water-bath and proceed the test with the residue as directed under the Flame Coloration Test (2): a green color is observed.

(4) The solution neutralized by adding dilute nitric acid to the water layer obtained in (3) responds to the Qualitative Tests for chloride.

Melting Point 192 ~ 196 °C.

pH Dissolve 1.0 g of Clomipramine Hydrochloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Clomipramine Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Clomipramine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Clomipramine Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Clomipramine Hydrochloride in exactly 10 mL of methanol and use this solution as the test solution. Separately, weigh 20 mg of Imipramine Hydrochloride RS, dissolve in methanol to make exactly 100 mL and use this solution as the standard solution (1). Then pipet 1 mL of the test solution and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 5 μL of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and ammonia solution (28) (15 : 5 : 1) to a distance of 10 cm and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots from the test solution corresponding to that from the standard solution (1) is not more intense than the spot from standard solution (1). Each of the spots other than the principal spot and the above spot from the test solution are not more intense than the spot from the standard solution (2).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.10 % (1 g).

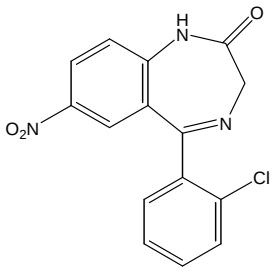
Assay Weigh accurately about 0.5 g of Clomipramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.131 mg of $C_{19}H_{23}ClN_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Clonazepam



$C_{15}H_{10}ClN_3O_3$: 315.71

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one [*1622-61-3*]

Clonazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of clonazepam ($C_{15}H_{10}ClN_3O_3$).

Description Clonazepam appears as white to pale yellow, crystals or crystalline powder.
Clonazepam is sparingly soluble in acetic anhydride or acetone, slightly soluble in methanol or in ethanol (95), very slightly soluble in ether and practically insoluble in water.
Clonazepam is gradually colored by light.

Melting point—About 240 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Clonazepam and Clonazepam RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Clonazepam and Clonazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clonazepam as directed under the Flame Coloration Test (2): a green color appears.

Purity (1) ***Chloride***—To 1.0 g of Clonazepam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. Discard the first 20 mL volumes of the filtrate, take the subsequent 20 mL volumes of the filtrate and add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution and perform the test. Prepare the control solution as follows: take 0.25ml of 0.01 mol/L hydrochloric acid VS and add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Clonazepam according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.25 g of Clonazepam in 10.0 mL of acetone and use this solution as the test solution. Pipet 1.0 mL of the test solution, add acetone to make exactly 100 mL, then pipet 1.0 mL of this solution, add acetone to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and acetone (10 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

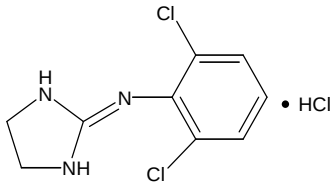
Assay Weigh accurately about 0.5 g of Clonazepam, previously dried, dissolve in 70 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.571 mg of $C_{15}H_{10}ClN_3O_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Clonidine Hydrochloride



$C_9H_9Cl_2N_3 \cdot HCl$: 266.56

N-(2,6-Dichlorophenyl)-4,5-dihydro-1*H*-imidazol-2-amine hydrochloride [*4205-91-8*]

Clonidine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$).

Description Clonidine Hydrochloride appears as white crystals or crystalline powder.
Clonidine Hydrochloride is very soluble in methanol, soluble in water or in ethanol (95), slighlty soluble in acetic acid (100) and practically insoluble in acetic

anhydride or ether.

Identification (1) To 5 mL of a solution of Clonidine Hydrochloride (1 in 1000), add 6 drops of Dragendorf's TS: an orange precipitate is produced.

(2) Determine the absorption spectra of solutions of Clonidine Hydrochloride and Clonidine Hydrochloride RS in 0.01 mol/L hydrochloride VS (3 in 10000), as directed under Ultraviolet-visible Spectrometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Clonidine Hydrochloride and Clonidine Hydrochloride RS, previously dried, as directed in potassium chloride disk method under Infrared Spectorphotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution (1 in 50) of Clonidine Hydrochloride responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—The solution of 1.0 g of Clonidine Hydrochloride in 20 mL of water, is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Clonidine Hydrochloride according to Method 1,.and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 0.5 g of Clonidine Hydrochloride, according to Method 3 and perform the test (not more than 4 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Clonidine Hydrochloride in 2.0 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution and add methanol to make exactly 100 mL. Take each 1.0 mL and 2.0 mL volumes of this solution, add methanol to make exactly 20 mL each and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot each 2 μL of the test solution and the standard solutions (1) and (2) on the plate of silica gel. Develop the plate with a mixture of toluene, 1,4-dioxane, ethanol (99.5), and ammonia solution (28) (10 : 8 : 2 : 1) to a distance of 12 cm and air-dry the plate. Dry the plate at 100 °C for 1 hour, spray evenly sodium hypochlorite TS and air-dry the plate for 15 minutes. Then, spray evenly potassium iodide-starch TS to the plate. The spots other than the principal and original spots are not more intense than the spot from the standard solution (2) and the number of spots, which are more intense than the spot from the standard solution (1), other than the principal and original spots is not more than 3.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

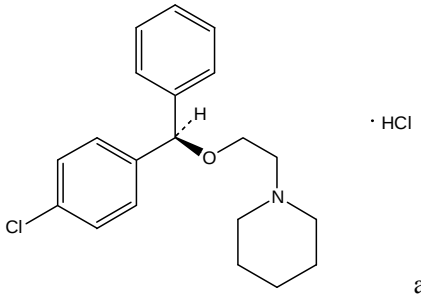
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.4 g of Clonidine Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100) and warm to dissolve. Cool, add 70 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.656 mg of $C_9H_9Cl_2N_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Cloperastine Hydrochloride



and enatiomer

$C_{20}H_{24}ClNO \cdot HCl$: 366.33

1-[2-[(4-Chlorophenyl)-
phenylmethoxy]ethyl]piperidine hydrochloride
[*14984-68-0*]

Cloperastine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of cloperastine hydrochloride ($C_{20}H_{24}ClNO \cdot HCl$).

Description Cloperastine Hydrochloride appears as white crystals or crystalline powder.
Cloperastine Hydrochloride is very soluble in water, in methanol, in ethanol (95) or in acetic acid (100), and sparingly soluble in acetic anhydride.
A solution of Cloperastine Hydrochloride (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Cloperastine Hydrochloride and Cloperastine Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared spectra of Cloperastine Hydrochloride and Cloperastine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spec-

tra exhibit similar intensities of absorption at the same wavenumbers.

(3) Shake 10 mL of a solution of Cloperastine Hydrochloride (1 in 100) with 2 mL of ammonia TS and 20 mL of ether, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Melting Point 148 ~ 152 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cloperastine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 40 mg of Cloperastine Hydrochloride in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1.0 mL of the test solution and add the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography, according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the areas of two peaks corresponding to the relative retention times of about 0.8 and 3.0 to the retention time of cloperastine obtained from the test solution are not larger than the peak area from the standard solution, respectively, and the area of the peak corresponding to the relative retention time of about 2.0 to cloperastine is not larger than 5/3 of the peak area from the standard solution, and the areas of the peaks other than cloperastine and other than the peaks mentioned above are all not larger than 3/5 of the peak area from the standard solution. The total area of these peaks is not larger than 2 times of the peak area of cloperastine from the standard solution .

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column, about 5 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol, 0.1 mol/L monobasic potassium phosphate TS and perchloric acid (500:250:1).

Flow rate: Adjust the flow rate so that the retention time of cloperastine is about 7 minutes.

System suitability

Selection of column: Dissolve 30 mg of Cloperastine Hydrochloride and 40 mg of benzophenone in 100 mL of the mobile phase. To 2.0 mL of this solution add the mobile phase to make 50 mL. Perform the test with 20 µL of this solution under the above operating conditions: Use a column giving elution of cloperastine and benzophenone in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cloperastine obtained from 20 µL of the standard solution is about 30 % of the full scale.

Time span of measurement: About 4 times as long as the retention time of cloperastine, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

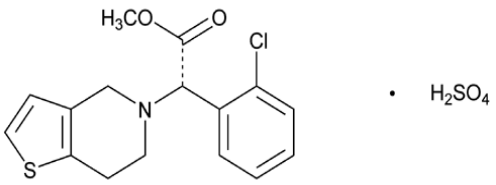
Assay Weigh accurately about 0.5 g of Cloperastine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.63 mg of $C_{20}H_{24}ClNO \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Clopidogrel Bisulfate



Clopidogrel Sulfate $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$: 419.90

Methyl (2S)-2-(2-chlorophenyl)-2-{4*H*,5*H*,6*H*,7*H*-thieno[3,2-c]pyridin-5-yl}acetate; sulfuric acid [*120202-66-6*]

Clopidogrel Bisulfate contains not less than 97.0 % and not more than 101.5 % of the labeled amount of clopidogrel bisulfate ($C_{16}H_{16}ClNO_2S \cdot H_2SO_4$: 419.90), calculated on the dried basis.

Description Clopidogrel Bisulfate appears as white powder.

Clopidogrel Bisulfate is freely soluble in water or in methanol, and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Clopidogrel Bisulfate and Clopidogrel Bisulfate RS as directed in the potassium bromide disk method under

Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(3) Clopidogrel Bisulfate responds to the Qualitative Tests for sulfate.

Purity ***Related substances***—Weigh accurately about 0.1 g of Clopidogrel Bisulfate, dissolve in 5 mL of methanol, add the mobile phase to make exactly 200 mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount each of Clopidogrel Bisulfate RS, Clopidogrel Related Substance I {(+)-(*S*)-(*o*-chlorophenyl)-6,7-dihydrothieno [3,2-*c*]pyridine-5(4*H*)-acetate} RS, Clopidogrel Related Substance II {methyl(±)-(*o*-chlorophenyl)4,5-dihydro-thieno[2,3-*c*]pyridine-6(7*H*)-acetate, hydrochloride} RS, and Clopidogrel Related Substance III {methyl(-)-(*R*)-(*o*-chlorophenyl)-6,7-dihydrothieno [3,2-*c*]pyridine-5(4*H*)-acetate, bisulfate} RS, and dissolve in methanol to dilute gradually so that each mL contains 20 μg, 40 μg, 120 μg, and 200 μg, respectively. To 5 mL of this solution add the mobile phase to make exactly 200 mL so that each mL contains 0.5 μg, 1 μg, 3 μg, and 5 μg, respectively, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances in the test solution: the amounts of clopidogrel related substance I, clopidogrel related substance III, and the first enantiomer of clopidogrel related substance II, having the relative retention times of about 0.5, about 2.0, and about 0.8 with respect to clopidogrel, are not more than 0.2 %, not more than 1.0 %, and not more than 0.3 %, respectively. The amount of each related substance is not more than 0.1 %, and the total amount of related substances is not more than 1.5 %.

$$\text{Amount (\%) of related substance I or III} = \frac{C_A}{C_T} \times \frac{A_U}{A_S} \times 100$$

C_A: Concentration (mg/mL) of each clopidogrel related substance in the standard solution

C_T: Concentration (mg/mL) of clopidogrel bisulfate in the test solution

A_U: Peak area of each related substance obtained from the test solution

A_S: Peak area of each clopidogrel related substance obtained from the standard solution

$$\text{Amount (\%) of the first enantiomer of related substance II} = \frac{C_B}{C_T} \times \frac{A_U}{A_S} \times 100 \times 0.5$$

C_B: Concentration (mg/mL) of each clopidogrel related substance II in the standard solution

C_T: Concentration (mg/mL) of clopidogrel bisulfate in the test solution

A_U: Peak area of the first enantiomer of clopidogrel related substance II obtained from the test solution

A_S: Peak area of the first enantiomer of clopidogrel related substance II obtained from the standard solution

0.5: Correction factor for the first enantiomer of clopidogrel related substance II

$$\text{Amount (\%) of each other related substance} = \frac{C}{C_T} \times \frac{A_U}{A_S} \times 100$$

C: Concentration (mg/mL) clopidogrel bisulfate in the standard solution

C_T: Concentration (mg/mL) of clopidogrel bisulfate in the test solution

A_U: Peak area of each related substance other than clopidogrel related substances I, II, and III obtained from the test solution

A_S: Peak area of clopidogrel related substances obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with silica gel for liquid chromatography bound to ovomucoid, a chiral-recognition protein, for liquid chromatography (5 μm in particle diameter).

Flow rate: 1.0 mL/minute

Mobile phase and system suitability solution: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 10 μL fo the system suitability solution under the above operating conditions, the relative retention times of the first and second enantiomers of clopidogrel related substance II with respect to clopidogrel are about 0.8 and about 1.2, respectively, and the resolution between the peaks of clopidogrel and the first enantiomer of clopidogrel related substance II is not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of each peak is not more than 15 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.1 g each of Clopidogrel Bisulfate and Clopidogrel Bisulfate RS, and add methanol to make exactly 100 mL each. Pipet 5.0 mL each

of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of clopidogrel bisulfate.

$$\text{Amount (mg) of clopidogrel bisulfate } (C_{16}H_{16}ClNO_2S \cdot H_2SO_4) = \text{Amount (mg) of Clopidogrel Bisulfate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with silica gel for liquid chromatography bound to ovomucoid, a chiral recognition protein, for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer solution and acetonitrile (75 : 25)

Phosphate buffer solution—Dissolve 1.36 g of potassium dihydrogen phosphate in 500 mL of water, and add water to make 1000 mL.

Flow rate: 1.0 mL/minute

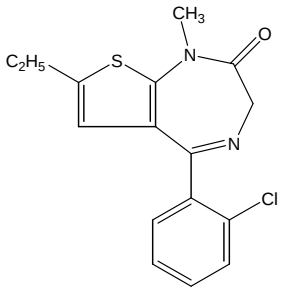
System suitability

System performance: Weigh accurately a suitable amount each of Clopidogrel Bisulfate RS and Clopidogrel Related Substance II RS, and dissolve in methanol so that each mL contains 100 μg and 200 μg, respectively. To 5 mL of this solution add the mobile phase to make 200 mL, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention times of the first and second enantiomers of clopidogrel related substance II with respect to clopidogrel are about 0.8 and about 1.2, respectively, and the resolution between the peaks of clopidogrel and the first enantiomer of clopidogrel related substance II is not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of clopidogrel is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Clotiazepam



$C_{16}H_{15}ClN_2OS$: 318.82

5-(2-Chlorophenyl)-7-ethyl-1-methyl-1*H*,2*H*,3*H*-thieno[2,3-e][1,4]diazepin-2-one [*33671-46-4*]

Clotiazepam, when dired, contains not less than 98.5 % and not more than 101.0 % of clotiazepam ($C_{16}H_{15}ClN_2OS$).

Description Clotiazepam appears as white to pale yellowish white crystals or crystalline powder, is odorless and has a slightly bitter taste.

Clotiazepam is very soluble in chloroform, freely soluble in methanol, in acetic acid (100), in ethanol (95), in acetone or in ethyl acetate, soluble in ether and practically insoluble in water.

Clotiazepam dissolves in 0.1 mol/L hydrochloric acid TS.

Clotiazepam is gradually colored by light.

Identification (1) Dissolve 10 mg of Clotiazepam in 3 mL of sulfuric acid: the solution shows a pale red fluorescence under ultraviolet light.

(2) Determine the absorption spectra of solutions of Clotiazepam and Clotiazepam RS in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra of absorption exhibit similar intensities at the same wavelengths.

(3) Prepare the test solution with 10 mg of Clotiazepam as directed under the Oxygen Flask Combustion Method, using 10 mL of diluted hydrogen peroxide water (30) (1 in 5) as the absorbing liquid. Apply a small amount of water to the upper part of the Apparatus A, pull out C carefully, wash C, B and the inner side of A with 15 mL of methanol and use the obtained solution as the test solution. Add 0.5 mL of dilute nitric acid to 15 mL of the test solution: this solution responds to the Qualitative Tests (2) for chloride. The remaining test solution responds to the Qualitative Tests (1) for sulfate.

Melting Point 106 ~ 109 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Clotiazepam in 10 mL of ethanol (95): the so-

lution is clear and has no more color than the following control solution.

Control solution—To 5 mL of Color Matching Fluid C, add 0.01 mol/L hydrochloric acid TS to make 10 mL.

(2) ***Chloride***—To 1.0 g of Clotiazepam, add 50 mL of water, shake for 30 minutes and filter. To 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Clotiazepam according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Clotiazepam, according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.25 g of Clotiazepam in 10.0 mL of acetone and use this solution as the test solution. Pipet 1.0 mL of the test solution, add acetone to make exactly 20 mL, pipet 2.0 mL of this solution, add acetone to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 80 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

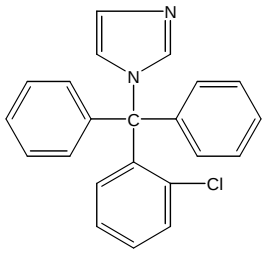
Assay Weigh accurately about 0.5 g of Clotiazepam, previously dried, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.882 mg of $C_{16}H_{15}ClN_2OS$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Clotrimazole



$C_{22}H_{17}ClN_2$: 344.84

1-[(2-Chlorophenyl)diphenylmethyl]-1*H*-imidazole
[*23593-75-1*]

Clotrimazole, when dried, contains not less than 98.0 % and not more than 101.0 % of clotrimazole ($C_{22}H_{17}ClN_2$).

Description Clotrimazole is a white, crystalline powder, is odorless and tasteless.
Clotrimazole is freely soluble in acetic acid (100) or in in dichloromethane, soluble in methanol, in ethanol (95), or in *N,N*-dimethylformamide, slightly soluble in ether and practically insoluble in water.

Identification (1) Dissolve 0.1 g of Clotrimazole in 10 mL of 5 mol/L hydrochloric acid TS by heating and cool. To this solution, add 3 drops of Reinecke salt TS: a pale red precipitate is produced.

(2) Determine the absorption spectra of solutions of Clotrimazole and Clotrimazole RS in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Clotrimazole and Clotrimazole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Clotrimazole as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 142 ~ 145 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Clotrimazole in 10 mL of dichloromethane: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 1.0 g of Clotrimazole in 40 mL of *N,N*-dimethylformamide, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.60 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of *N,N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021 %).

(3) ***Sulfate***—Dissolve 0.5 g of Clotrimazole in 10 mL of methanol and add 1 mL of dilute hydrochloric

acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.05 mol/L sulfuric acid VS, 10 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Clotrimazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Clotrimazole according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Imidazole***—Dissolve 0.10 g of Clotrimazole in exactly 10 mL of dichloromethane and use this solution as the test solution. Separately, dissolve 25.0 mg of imidazole RS in dichloromethane to make exactly 50 mL. Pipet 5.0 mL of this solution, add dichloromethane to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and chloroform (3 : 2) to a distance of about 10 cm and air-dry the plate. Spray evenly sodium hypochlorite TS on the plate and air-dry the plate for 15 minutes, then spray evenly potassium iodide-starch TS on the plate: the spot from the test solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

(7) ***(2-Chlorophenyl)-diphenylmethanol***—Dissolve 0.20 g of Clotrimazole in dichloromethane to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 10.0 mg of (2-chlorophenyl)-diphenyl-methanol RS in dichloromethane to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (50 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.35 g of Clotrimazole, previously dried and dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.484 mg of $C_{22}H_{17}ClN_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cloxacillin Sodium Capsules

Cloxacillin Sodium Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$: 435.88).

Method of Preparation Prepare as directed under Capsules, with Cloxacillin Sodium.

Identification (1) Put an amount of the contents of Cloxacillin Sodium Capsules, equivalent to 2 mg of cloxacillin sodium, in a test tube, add 2 mg of chromotropic acid and 2 mL of sulfuric acid, and heat at 150 °C: a green color develops in 1 to 1.5 minutes, changes to deep red after 2 minutes, and gradually becomes dark black as it carbonizes.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 capsule of Cloxacillin Sodium Capsules at 100 revolutions per minute according to Method 1, using 900 mL of 0.05 mol/L potassium dihydrogen phosphate solution adjusted to pH 6.8 as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Cloxacillin Sodium RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cloxacillin sodium in each solution. The dissolution rate of Cloxacillin Sodium Capsules in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of cloxacillin sodium ($C_{19}H_{18}ClN_3O_5S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in 1 capsule

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: A mixture of the buffer solution and acetonitrile (80 : 20)

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of cloxacillin is not more than 1.8.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cloxacillin is not more than 2.0 %.

Buffer solution—Adjust the pH of 0.02 mol/L potassium dihydrogen phosphate solution to 6.8 with 2 mol/L sodium hydroxide.

Uniformity of Dosage Units It meets the requirement.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately the mass of contents of not less than 20 Cloxacillin Sodium Capsules. Weigh accurately a portion of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Cloxacillin Sodium RS, proceed in the same manner as for the test solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cloxacillin sodium in each solution.

$$\text{Amount [μg (potency)] of cloxacillin } (C_{19}H_{18}ClN_3O_5S) = \text{Amount [μg (potency)] of Cloxacillin Sodium} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

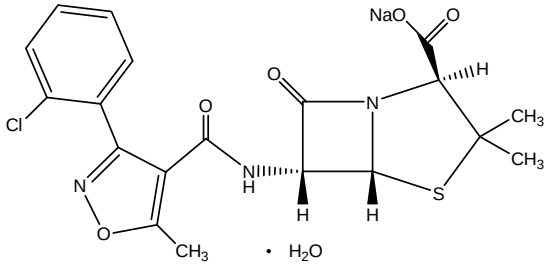
Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 1000 mL of a solution prepared by dissolving 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL and adjusting the pH to 5.0 with 8 mol/L potassium hydroxide solution, and 500 mL of acetonitrile

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Cloxacillin Sodium Hydrate



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$: 475.88

Sodium (2*S*,5*R*,6*R*)-6-[[3-(2-chlorophenyl)-5-methyl-2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylatehydrate [*7081-44-9*]

Cloxacillin Sodium Hydrate contains not less than 900 μg (potency) and not more than 960 μg (potency) per mg of cloxacillin ($C_{19}H_{17}ClN_3NaO_5S$: 435.88), calculated on the anhydrous basis.

Description Cloxacillin Sodium Hydrate appears as whtie to light yellowish white crystals or crystalline powder.

Cloxacillin Sodium Hydrate is freely soluble in water, in *N,N*-dimethylformamide, or in methanol, and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Cloxacillin Sodium Hydrate and Cloxacillin Sodium RS in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Cloxacillin Sodium Hydrate and Cloxacillin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Cloxacillin Sodium Hydrate responds to the Qualitative Tests for sodium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +163 ~ +171° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 mg of Cloxacillin Sodium Hydrate in 10 mL of water is between 5.0 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water: the solution is clear and colorless to light yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Cloxacillin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Cloxacillin Sodium Hydrate according to Method 5, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately 50 mg of Cloxacillin Sodium Hydrate, dissolve in the mobile phase to make 50 mL, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than cloxacillin from the test solution is not larger than the peak area of cloxacillin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 4.953 g of diammonium hydrogen phosphate in 700 mL of water, and add 250 mL of acetonitrile. Adjust the pH of this solution to 4.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cloxacillin is about 24 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cloxacillin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: Weigh accurately about 50 mg of Cloxacillin Sodium RS, dissolve in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), add the mobile phase to make exactly 50 mL, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cloxacillin to that of guaifenesin is not more than 1.0 %.

Time span of measurement: About 3 times as long as the retention time of cloxacillin

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Cloxacillin Sodium Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the supernatant as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS, add 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Cloxacillin Sodium Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water 3.0 ~ 4.5 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Cloxacillin Sodium Hydrate is used in a sterile prepara-

tion.

Bacterial Endotoxins Less than 0.20 EU/mg of cloxacillin, when Cloxacillin Sodium Hydrate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Cloxacillin Sodium Hydrate and Cloxacillin Sodium Hydrate RS, dissolve in water to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T, and A_S, of cloxacillin in each solution.

$$\text{Amount [μg (potency)] of cloxacillin } (C_{19}H_{17}ClN_3NaO_5S) = \text{Amount [μg (potency)] of Cloxacillin Sodium Hydrate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

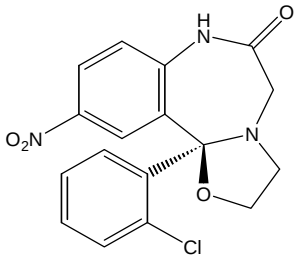
Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 5.0 with 8 mol/L potassium hydroxide solution. To 1000 mL of this solution, add 500 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

Containers and Storage ***Containers***—Tight containers.

Cloxazolam



and enantiomer

$C_{17}H_{14}Cl_2N_2O_2$: 349.21

10-Chloro-11b-(2-chlorophenyl)-2,3,7,11b-tetrahydrobenzo[f]oxazolo[3,2-d][1,4]diazepin-6(5*H*)-one [*24166-13-0*]

Cloxazolam, when dried, contains not less than 99.0 % and not more than 101.0 % of cloxazolam ($C_{17}H_{14}Cl_2N_2O_2$).

Description Cloxazolam appears as white crystals or crystalline powder, is odorless and tasteless.

Cloxazolam is freely soluble in acetic acid (100), sparingly soluble in dichloromethane, slightly soluble in dehydrated ethanol or in ether, very slightly soluble in ethanol and practically insoluble in water.

Cloxazolam dissolves in dilute hydrochloric acid.

Cloxazolam is gradually colored by light.

Melting point—About 200 °C (with decomposition).

Identification (1) Dissolve 10 mg of Cloxazolam in 10 mL of dehydrated ethanol by heating and add 1 drop of hydrochloric acid: the solution shows a pale yellow color and a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 10 mg of Cloxazolam in 5 mL of dilute hydrochloric acid by heating in a water-bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests for primary aromatic amines.

(3) Place 2 g of Cloxazolam in a flask, add 50 mL of ethanol and 25 mL of sodium hydroxide TS and boil under a reflux condenser for 4 hours. After cooling, neutralize with dilute hydrochloric acid and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter and evaporate the dichloromethane of the filtrate. Dissolve the residue in 5 mL of methanol by heating in a water-bath and cool immediately in an ice-bath. Collect the crystals and dry the crystals in vacuum at 60 °C for 1 hour: it melts between 87 °C and 91 °C.

(4) Determine the absorption spectra of solutions of Cloxazolam and Cloxazolam RS in ethanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities at the same wavelengths.

(5) Proceed with Cloxazolam as directed under the Flame Coloration Test (2): a green color appears.

Absorbance $E_{1\,cm}^{1\%}$ (244 nm): 390 ~ 410 (after drying, 1 mg, anhydrous ethanol, 100 mL).

Purity (1) ***Chloride***—To 1.0 g of Cloxazolam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. To 25 mL of this filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Cloxazolam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Place 1.0 g of Cloxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid and heat gently. Repeat the addition of 2 mL to 3 mL of nitric acid at times and continue heating until a colorless to pale yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate solution and heat the solution until dense white fumes are evolved and evaporate to a volume of 2 mL to 3 mL. After cooling, dilute with water to make 10 mL and perform the test with this solution as the test solution (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50.0 mg of Cloxazolam in 10.0 mL of dichloromethane and use this solution as the test solution. Pipet 1.0 mL of this solution, add dichloromethane to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of toluene and acetone (5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

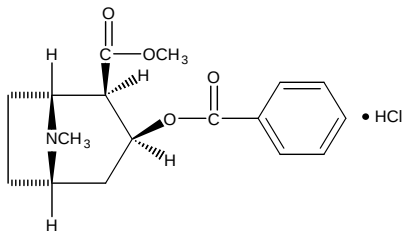
Assay Weigh accurately about 0.5 g of Cloxazolam, previously dried and dissolve in 50 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.921 mg of $C_{17}H_{14}Cl_2N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cocaine Hydrochloride



$C_{17}H_{21}NO_4$·HCl: 339.81

Methyl (1*R*,3*R*,4*S*,5*S*)-3-Benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylate hydrochloride [*53-21-4*]

Cocaine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of cocaine hydrochloride ($C_{17}H_{21}NO_4$·HCl).

Description Cocaine Hydrochloride appears as colorless crystals or white crystalline powder.
Cocaine Hydrochloride is very soluble in water, freely soluble in ethanol (95) or in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in ether.

Identification (1) Determine the absorption spectra of solutions of Cocaine Hydrochloride and Cocaine Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. Determine the absorption spectra of solutions of Cocaine Hydrochloride and Cocaine Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 50000) as directed under the Ultraviolet-visible spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths

(2) Determine the infrared spectra of Cocaine Hydrochloride and Cocaine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the similar intensities at the same wavenumbers.

(3) A solution of Cocaine Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chlorides.

Specific Optical Rotation $[\alpha]_D^{20}$: -70 ~ -73° (after drying, 0.5 g, water, 20 mL, 100 mm).

Purity (1) ***Acidity***—Dissolve 0.5 g of Cocaine Hydrochloride in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.01 mol/L sodium hydroxide VS: not more than 1.0 mL is required to obtain a yellow color.

(2) ***Cinnamylcocaine***—Dissolve 0.10 g of Cocaine Hydrochloride in 5 mL of water, and add 0.3 mL of diluted sulfuric acid (1 in 20) and 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color does not disappear entirely within 30 minutes.

(3) ***Isoatropy cocaine***—Dissolve 0.10 g of Cocaine Hydrochloride in 30 ml of water in a beaker. Transfer 5 ml of this solution to a test tube, add 1 drop of ammonia TS, and mix. After the precipitate is coagulated, add 10 ml of water, and transfer the mixture to the former beaker, to which 30 ml of water has been added previously. Wash the test tube with 10 ml of water, combine the washing with the mixture in the beaker, add 3 drops of ammonia TS to the combined mixture, and mix gently: a crystalline precipitate is produced.

Allow to stand for 1 hour: the supernatant liquid is clear.

(4) ***Readily carbonizable substances***—Perform the test with 0.5 g of Cocaine Hydrochloride: the solution has no more color than Color Matching Fluid F.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately 0.5 g of Cocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.982 mg of $C_{17}H_{21}NO_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cod Liver Oil

Cod Liver Oil is the fatty oils obtained from fresh livers and pyloric appendages of Gadus macrocephalus Tilesius or Theraga chalcogramma Pallas (Gadidae).
Cod Liver Oil contains not less than 2000 vitamin A units and not more than 5000 vitamin A units per g.

Description Cod Liver Oil is a yellow to orange oily liquid, has a characteristic, slightly fishy odor and a mild taste.
Cod Liver Oil is miscible with chloroform.
Cod Liver Oil is slightly soluble in ethanol (95) and practically insoluble in water.
Decomposition of Cod Liver Oil is accelerated by air or by light.

Identification Dissolve 0.1 g of Cod Liver Oil in 10 mL of chloroform, and to 1 mL of this solution, add 3 mL of antimony (III) chloride TS: a blue color is observed immediately, but the color disappears rapidly.

Specific Gravity d_{20}^{20} : 0.918 ~ 0.928.

Acid Value Not more than 1.7.

Saponification Value 180 ~ 192.

Unsaponifiable Matter Not more than 3.0 %

Iodine Value 130 ~ 170.

Anisidine Value Weigh accurately 0.5 g of Cod Liver Oil, dissolve in isooctane to make exactly 25 mL and use this solution as the test stock solution. Pipet 5 mL of this solution, add exactly 1 mL of acetic acid (100) solution (2.5 in 1000) of *p*-anisidine, shake and use this solution as the test solution. Separately, take 5.0 mL of isooctane, add exactly 1 mL of acetic acid (100) solution (2.5 in 1000) of *p*-anisidine, shake and use this solution as the standard solution. Store the test solution and the standard solution under protection from light. Measure the absorbance of the test stock solution A_{S1} at 350 nm, using isooctane as the blank solution. Measure the absorbance of the test solution A_{S2} at 350 nm exactly 10 minutes after making the test solution, using the standard solution as the blank. And calculate the anisidine value according to the following equation: not more than 30.

$$\text{Anisidine value} = 25 \times \frac{(1.2 \times A_{S2}) - A_{S1}}{\text{Weight(g) of Cod Liver Oil taken}}$$

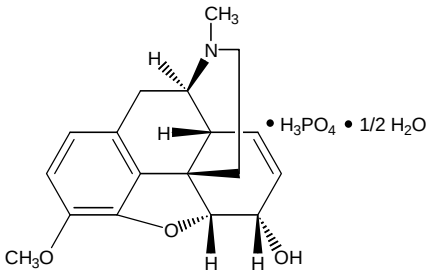
Purity ***Rancidity***—No unpleasant odor of rancid oil is perceptible on warming Cod Liver Oil.

Assay Perform the test with about 0.5 g of Cod Liver Oil, weighed accurately, as directed in Method 2 under the Vitamin A Determination.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and almost well-filled or under nitrogen atmosphere.

Codeine Phosphate Hydrate



Codeine Phosphate

$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$: 406.37

(5*R*,6*S*)-4,5-Epoxy-3-methoxy-17-methyl-7,8-didehydromorphinan-6-ol monophosphate hemihydrate [*41444-62-6*]

Codeine Phosphate Hydrate contains not less than 98.0 % and not more than 101.0 % of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4$: 397.36), calculated on the anhydrous basis.

Description Codeine Phosphate Hydrate appears as white to yellowish white crystals or crystalline powder. Codeine Phosphate Hydrate is freely soluble in water or in acetic acid (100), slightly soluble in methanol or in ethanol (95) and practically insoluble in ether.

pH—A solution of Codeine Phosphate Hydrate (1 in 10) is between 3.0 and 5.0.

Codeine Phosphate Hydrate is affected by light.

Identification (1) Determine the absorption spectra of solutions of Codeine Phosphate Hydrate and Codeine Phosphate Hydrate RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Codeine Phosphate Hydrate and Codeine Phosphate Hydrate RS, previously dried at 105 °C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Codeine Phosphate Hydrate (1 in 20) responds to the Qualitative Tests (1) for phosphate.

Specific Optical Rotation $[\alpha]_D^{20}$: -98 ~ -102° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity (1) ***Chloride***—Perform the test with 0.5 g of Codeine Phosphate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(2) ***Sulfate***—Perform the test with 0.20 g of Codeine Phosphate Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240 %).

(3) ***Related substances***— Dissolve 0.20 g of Codeine Phosphate Hydrate in 10 mL of a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14 : 14 : 7 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water 1.5 ~ 3.0 % (0.5 g, volumetric titration, direct titration).

Asssy Dissolve about 0.5 g of Codeine Phosphate Hydrate, accurately weighed, in 70 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.736 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

1 % Codeine Phosphate Powder

1 % Codeine Phosphate Powder contains not less than 0.90 % and not more the 1.10 % of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$: 406.37).

Method of Preparation

Codeine Phosphate Hydrate	10 g
Lactose hydrate	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1 % Codeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 283 and 287 nm.

Particle Size Distribution Test for preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately about 5 g of 1 % Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and use this solution as the test solution. Previously, weigh accurately about 50 mg of Codeine Phosphate RS, separately determined the water content in the same manner as Codeine Phosphate Hydrate, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard for the test solution and

the standard solutions, respectively.

$$\text{Amount (mg) of codeine phosphate hydrate } (C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2\ H_2O) = \text{Amount (mg) of Codeine Phosphate RS, calculated on the anhydrous basis} \times 1.0227 \times \frac{Q_T}{Q_S}$$

Internal Standard soultion—A solution of etilefline hydrochloride (3 in 10000).

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay under 10 % Codeine Phosphate Powder.

Containers and Storage ***Containers***—Tight containers.

10 % Codeine Phosphate Powder

10 % Codeine Phosphate Powder contains not less than 9.3 % and not more than 10.7 % of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$: 406.37).

Method of Preparation

Codeine Phosphate hydrate	100 g
Lactose hydrate	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Test Determine the absorption spectrum of a solution of 10 % Codeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 283 and 287 nm.

Particle Size Distribution Test for preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement

Assay Weigh accurately about 2.5 g of 10 % Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20.0 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Codeine Phosphate Hydrate RS, previously determined the water content in the same manner as Codeine Phosphate Hydrate, dissolve in water to make exactly 100 mL, then pipet 10.0 mL of this solution, add exactly 10 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of codeine phosphate hydrate } (C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2\ H_2O) = \text{Amount (mg) of Codeine Phosphate Hydrate RS, calculated on the anhydrous basis} \times 1.0227 \times \frac{Q_T}{Q_S} \times 5$$

Internal standard solution—A solution of etilefline hydrochloride (3 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000) and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution, add 70 mL of tetrahydrofuran and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution according to the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard substance is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Codeine Phosphate Tablets

Codeine Phosphate Tablets contains not less than 93.0 % and not more than 107.0 % of labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$: 406.37).

Method of Preparation Prepare as directed under

Tablets, with Codeine Phosphate Hydrate.

Identification To a portion of powdered Codeine Phosphate Tablets, equivalent to 0.1 g of Codeine Phosphate Hydrate according to the labeled amount, add 20 mL of water, shake and filter. To 2 mL of the filtrate, add water to make 100 mL and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 283 and 287 nm.

Dissolution Test Perform the test with 1 tablet of Codeine Phosphate Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Codeine Phosphate RS (previously determine the water in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under Liquid Chromatography, and determine the peak areas, A_T and A_S, of codeine in each solution. The dissolution rate of Codeine Phosphate Tablets in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18 \times 1.0227$$

W_S: Amount (mg) of Codeine Phosphate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$) in 1 tablet

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of codeine is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Codeine Phosphate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$), add 30 mL of water, sonicate for 10 minutes, and add water to make exactly 100 mL. Filter the solution, then pipet 5 mL of the filtrate, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Codeine Phosphate Hydrate RS (previously determine the water in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard.

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 1 \cdot /2H_2O$)

$$= W_S \times \frac{Q_T}{Q_S} \times 2 \times 1.0227$$

W_S : Amount (mg) of Codeine Phosphate Hydrate RS, calculated on the anhydrous basis

Internal standard solution— A solution of etilefline hydrochloride (3 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability

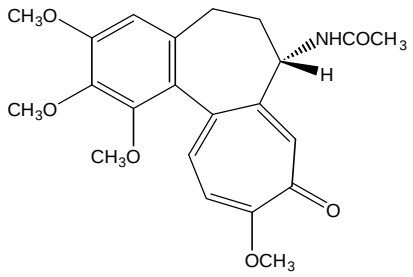
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard

are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Colchicine



$C_{22}H_{25}NO_6$: 399.44

N-{3,4,5,14-Tetramethoxy-13-oxotricyclo [9.5.0.0^{2,7}]hexadeca-1(16),2(7),3,5,11,14-hexaen-10-yl}acetamide [*64-86-8*]

Colchicine, when dired, contains not less than 97.0 % and not more than 102.0 % of colchicine ($C_{22}H_{25}NO_6$), calculated on the anhydrous basis and corrected by the amount of ethyl acetate.

Description Colchicine is a yellowish white powder.
Colchine is very soluble in methanol, freely soluble in ethanol (95), in acetic anhydride, or in *N,N*-dimethylformamide, and sparingly soluble in water.
Colchicine is colored by light.

Identification (1) Determine the absorption spectra of solutions of Colchicine and Colchicine RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities absorption at the same wavelength.

(2) Add each 0.5 mL of solutions of Colchicine and Colchicine RS in methanol (1 in 50) to 1 g of potassium bromide for infrared absorption spectrum, grind thoroughly, dry in vacuum at 80 °C for 1 hour, determine the absorption spectra of these powders as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -235 ~ -250° (0.1 g calculated on the anhydrous basis and corrected by the amount of ethyl acetate, ethanol (95), 10 mL, 100 mm)

Purity (1) ***Colchiceine***— Dissolve 0.1 g of Colchicine in 10 mL of water and to 5 mL of this solution, add 2 drops of iron (III) chloride TS: no definite green color is produced.

(2) ***Ethyl acetate and Chloroform*** —Weigh accurately 0.6 g of Colchicine, dissolve in exactly 2 mL of internal standard solution, and add *N,N*-dimethylformamide to make 10 mL, and use this solution as the test solution. Separately, weigh 0.3 g of chloroform using the 100 mL-volumetric flask containing about 20 mL of *N,N*-dimethylformamide, add *N,N*-dimethylformamide to make 100 mL. Pipet 2 mL of this solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution (1). Separately weigh accurately about 1.8 g of ethyl acetate, using the 100-mL volumetric flask containing about 20 mL of *N,N*-dimethylformamide, add *N,N*-dimethylformamide to make 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of internal standard solution, add *N,N*-dimethyl-formamide to make 10 mL, and use this solution as the standard solution (2). Perform the test with each 2 μL of the test solution, the standard solution (1) and the standard solution (2), as directed under Gas Chromatography according to the following conditions: the peak area of chloroform obtained from the test solution is not more than the that obtained from the standard solution (1). Determine the ratios of peak area, Q_T and Q_S, of ethyl acetate to the peak area of the internal standard obtained from the test solution and the standard solution. Calculate the amount of ethyl acetate according to the following equation: the amount of ethylacetate is not more than 6.0 %.

$$\text{Amount (mg) of ethyl acetate} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times 2$$

W_S: Amount (g) of ethylacetate
W_T: Amount (g) of Colchicine

Internal standard solution—A solution of 1-propanol in *N,N*-dimethylformamide (3 in 200).

Operating conditions

Detector: A hydrogen flame ionized detector

Column: A fused glass column, about 0.53 mm in internal diameter and about 30 m in length, coated inside surface with polyethylene glycol 20 M for gas chromatograph 1.0 μm in thickness.

Column temperature: 60 °C for 7 minutes then up to 100 °C at a rate of 40 °C per minute if necessary, and hold at 100 °C for 10 minutes.

Inject port temperature: A constant temperature of about 130 °C.

Detector temperature: A constant temperature of about 200 °C.

Carrier gas: Helium

Flow rate: Adjust the folw rate so that the retention

time of ethylacetate is about 3 minutes.

Split ratio : about 1 : 20

System suitability

Test for required detactability: Pipet 2 mL of the standard solution (2), and add *N,N*-dimethylformamide to make exactly 25 mL. Pipet 1 mL of this solution, and add *N,N*-dimethylformamide to make exactly 50 mL. Confirm that the peak area of ethylacetate obtained from 2 μL of this solution is equivalent to 0.11 and 0.21 % of that obtained from 2μL of the standard solution(2).

System performance: To 1 mL of chloroform add *N,N*-dimethylformamide to make 10 mL. To 1 mL of this solution add 2 mL of ethylacetate, and *N,N*-dimethylformamide to make 10 mL. To 2 mL of this solution add 2 mL of the internal standard solution and *N,N*-dimethylformamide to make 10 mL. When the procedure is run with 2 μL of this solution according to the above operation conditions, ethylacetate, chloroform and internal standard are eluted in this order, with the resolution between chloroform and internal standard being not less than 2.0.

System repeatability: When the test is repeated 3 times with 2 μL each of the standard solution (2) according to the above operating conditions, the relative standard deviation of the ratio of peak area of ethylacetate to that of the internal is not more than 3.0 %.

(3) ***Related substances***—Dissolve 60 mg of Colchicine in 100 mL of diluted methanol (1 in 2). Pipet 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution by the automatic integration method and calculate the total amount of the peaks other than colchicines by the area percentage method: not more than 5.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column,about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Add methanol to 450 mL of 0.05 mol/L potassium dihydrogen phosphate TS to make 1000 mL, Adjust pH to 5.5 with diluted phosphoric acid (7 in 200) .

Flow rate: Adjust the flow rate so that the retention time of colchicines is about 7 minutes.

System suitability

Test for required detactability: Pipet 1 mL of the test solution, and add diluted methanol (1 in 2) to make exactly 50 mL. Confirm that the peak area of colchicine obtained from 20 μL of this solution is equivalent to 1.4 to 2.6 % of that from the test solution.

System performance: When the procedure is run with 20 μL of the test solution according to the above operating conditions, the number of theoretical plates is 6000, and the symmetry factor is not more than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the test solution according to the above operation conditions, the relative standard deviation of the peak area of colchicine is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of colchicine beginning after the peak of the solvent.

Water Not more than 2.0 % (0.5 g, volumetric titration method, back titration)

Assay Weigh accurately 0.4 g of Colchicine, previously dried, dissolve in 25 mL of acetic anhydride and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 19.97 mg of $C_{22}H_{25}NO_6$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Colchicine Tablets

Colchicine Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of colchicine ($C_{22}H_{25}NO_6$: 399.44).

Preparation Prepare as directed under Tablets, with Colchicine.

Identification Weigh a portion of powdered Colchicine Tablets, equivalent to 20 mg of Colchicine, add 20 mL of water, allow the solids to settle and filter the clear supernatant liquid into a separatory funnel. Extract the filtrate with 30 mL of chloroform and evaporate the chloroform extract to dryness. Determine the infrared spectra of the residue and Colchicine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Dissolution Test Perform the test with 1 tablet of Colchicine Tablets at 100 revolutions per minutes according to Method 1 under the Dissolution Test, using 500 mL of water as a dissolution solution. Filter the dissolved solution through a membrane filter with a pore size of not more than 0.8 μm, after 30 minutes from the start of the test, pipet *V* mL of the filtrate and

extract with 15 mL of chloroform three times. Evaporate the combined extracts to dryness, add chloroform to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately a portion of Colchicine RS, previously dried at 105 °C for 3 hours, dissolve in dissolution solution, prepare the same concentration of solution as the test solution and use this solution as the standard solution. Determine the absorbance of the test solution and the standard solution at 350 nm as directed under Ultraviolet-visible Spectrophotometry using chloroform as a blank.
The dissolution rate of Colchicine Tablets in 30 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Colchicine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.6 mg of colchicine ($C_{22}H_{25}NO_6$), add a mixture of methanol and water (1 : 1), shake by mechanical means for 15 minutes, dilute with the same mixture to make exactly 100 mL, filter and use this solution as the test solution (prepared immediately prior to use). Separately, weigh accurately a portion of Colchicine RS, previously dried at 105 °C for 3 hours, dissolve in a mixture of methanol and water (1 : 1) and use this solution as the standard solution so that each mL contains 6 μg of Colchicine. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas, A_T and A_S, of colchicine of the test solution and the standard solution, respectively.

$$\text{Amounts (mg) of colchicine } (C_{22}H_{25}NO_6) = 0.1 \times C \times \frac{A_T}{A_S}$$

C: Concentration (g/mL) of the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Add 45 mL of 0.5 mol/L potassium dihydrogen phosphate and water to make 450 mL. Add 530 mL of methanol to this solution, cool to room temperature and dilute with methanol to make 1000 mL. Adjust pH to 5.5 ± 0.05 with 0.5 mol/L phosphoric acid.

Flow rate: 1 mL/minute.

System suitability

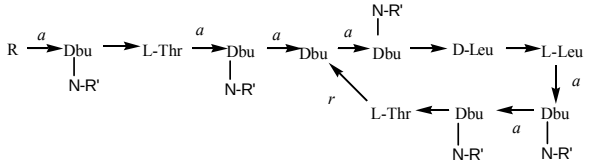
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the retention time of colchicine is between 5.5 and 9.5 minutes, and the number of theoretical plates is not less than 4500.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of colchicine is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Colistin Sodium Methanesulfonate



Colistin A: R = 6-Methyloctanoic acid
R' = Sodium methanesulfonate
Colistin B: R = Methylheptanoic acid
R' = Sodium methanesulfonate
Dbu: L-*α,γ*-Diaminobutyric acid
Thr: Threonine Leu: Leucine

$C_{58}H_{105}N_{16}Na_5O_{28}S_5$ (Colistin A): 1749.82
$C_{57}H_{105}N_{16}Na_5O_{28}S_5$ (Colistin B): 1735.80

Colistin A
Pentasodium [2-[(2*S*,5*R*,8*S*,11*S*,14*S*,17*S*,22*S*)-17-[(1*R*)-1-hydroxyethyl]-22-[[(2*S*)-2-[[(2*S*,3*R*)-3-hydroxy-2-[[(2*S*)-2-[[(6*R*)-6-methyloctanoyl]amino]-4-(sulfonatomethylamino)butanoyl]amino]butanoyl]amino]-4-(sulfonatomethylamino)butanoyl]amino]-5,8-*bis*(2-methylpropyl)-3,6,9,12,15,18,23-heptaoxo-11,14-*bis*[2-(sulfonatomethylamino)ethyl]-1,4,7,10,13,16,19-heptazacyclotricos-2-yl]ethylamino]methanesulfonate
Colistin B
Pentasodium [2-[(2*S*,5*R*,8*S*,11*S*,14*S*,17*S*,22*S*)-17-[(1*R*)-1-hydroxyethyl]-22-[[(2*S*)-2-[[(2*S*,3*R*)-3-hydroxy-2-[[(2S)-2-(6-methylheptanoylamino)-4-(sulfonatomethylamino)butanoyl]amino]butanoyl]amino]-4-(sulfonatomethyl-amino)butanoyl]amino]-5,8-*bis*(2-methylpropyl)-3,6,9,12,15,18,23-heptaoxo-11,14-*bis*[2-(sulfonatomethylamino)ethyl]-1,4,7,10,13,16,19-heptazacyclotricos-2-yl]ethylamino]methanesulfonate [*8068-28-8*]

Colistin Sodium Methanesulfonate is the sodium salt of colistin derivatives, and is a mixture of colistin A sodium methanesulfonate and colistin B sodium methanesulfonate.
Colistin Sodium Methanesulfonate, when dried, contains not less than 11500 units per mg. The potency of Colistin Sodium Methanesulfonate expresses the

amount of colistin A (R=6-methyloctanoic acid, R′=H, $C_{53}H_{100}N_{16}O_{13}$: 1169.46) in units.

Description Colistin Sodium Methanesulfonate appears as white to pale yellowish white powder.
Colistin Sodium Methanesulfonate is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) Dissolve 20 mg of Colistin Sodium Methanesulfonate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, and add 5 drops of copper (II) sulfate ts while shaking: a blue-purple color develops.

(2) Dissolve 40 mg of Colistin Sodium Methanesulfonate in 1 mL of hydrochloric acid TS, and add 0.5 mL of dilute iodine TS: the color of iodine disappears.

(3) Determine the infrared spectra of Colistin Sodium Methanesulfonate and Colistin Sodium Methanesulfonate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Colistin Sodium Methanesulfonate responds to the Qualitative Tests (1) for sodium salt.

pH Dissolve 0.1 g of Colistin Sodium Methanesulfonate in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.5 and 8.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.16 g of Colistin Sodium Methanesulfonate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test (not more than 2 ppm).

(4) ***Free colistin***—Dissolve 80 mg of Colistin Sodium Methanesulfonate in 3 mL of water, add 0.05 mL of a solution of silicotungstic acid 26-water (1 in 10), and immediately compare the solution with the reference suspension described under Test Metods for Plastic Containers: the turbidity is not greater than that of the reference suspension (not more than 0.25 %).

Loss on Drying Not more than 3.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Colistin Sodium Methanesulfonate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.67 EU/10000 units of colistin, when Colistin Sodium Methanesulfonate is used in a sterile preparation.

Assay ***The Cylinder-plate method*** (1) Test organism- *Escherichia coli* NIHJ

(2) Culture medium- To 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of beef extract, and 20.0 g of agar, add 1000 mL of water, adjust the pH of the medium with sodium hydroxide TS so that it will be 6.5 to 6.6 after sterilization, sterilize, and use as the agar media for seed and base layer.

(3) Weigh accurately a suitable amount of Colistin Sodium Methanesulfonate, previously dried, dissolve in phosphate buffer solution (pH 6.0) so that each mL contains about 100000 units, and use this solution as the test stock solution. Pipet a suitable volume of the test stock solution, add phosphate buffer solution (pH 6.0) so that each mL contains 10000 units and 2500 units, and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately a suitable amount of Colistin Sodium Methanesulfonate RS, previously dried, dissolve in phosphate buffer solution (pH 6.0) so that each mL contains 100000 units, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 10 °C, and use within 7 days. Pipet a suitable volume of the standard stock solution, add phosphate buffer solution (pH 6.0) so that each mL contains 10000 units and 2500 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Colistin Sodium Methanesulfonate for Injection

Colistin Sodium Methanesulfonate for Injection is a preparation for injection, which is dissolved before use. Colistin Sodium Methanesulfonate contains not less than 90.0 % and not more than 120.0 % of the labeled amount of colistin.

Method of Preparation Prepare as directed under Injections, with Colistin Sodium Methanesulfonate.

Description Colistin Sodium Methanesulfonate for Injection appears as white to pale yellowish white powder.

Identification Proceed as directed in Identification under Colistin Sodium Methanesulfonate.

pH Dissolve 0.1 g of Colistin Sodium Methanesulfonate for Injection in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.0 and 8.0.

Purity ***Heavy metals***—Proceed with 1.0 g of Colistin Sodium Methanesulfonate for Injection according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.67 EU/10000 units of Colistin Sodium Methanesulfonate for Injection.

Foreign Insoluble Matter Test It meets the requirement.

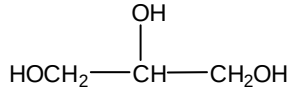
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate Method*** Proceed as directed in the Assay under Colistin Sodium Methanesulfonate. Weigh accurately a suitable amount of Colistin Sodium Methanesulfonate for Injection, and add 1 % phosphate buffer solution (pH 6.0) to make a solution so that each mL contains 100000 units (potency). Pipet a suitable volume of this solution, add 1 % phosphate buffer solution (pH 6.0) so that each mL contains 10000 units and 2500 units, and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers.

Concentrated Glycerin



Concentrated Glycerol $C_3H_8O_3$: 92.09

Concentrated Glycerin contains not less than 98.0 % and not more than 101.0 % of glycerin ($C_3H_8O_3$), calculated on the anhydrous basis.

Description Concentrated Glycerin is a clear, colorless and viscous liquid and has a sweet taste.
Concentrated Glycerin is miscible with water or with ethanol (95).
Concentrated Glycerin is hygroscopic.

Identification (1) Determine the infrared spectra of Concentrated Glycerin and Glycerin RS as directed in the liquid film method under Infrared Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The retention of Glycerin from the test solution under Purity (10) corresponds to that of the standard preparation.

Refractive index n_D^{20} : Not less than 1.470

Specific Gravity d_{20}^{20} : Not less than 1.258.

Purity (1) ***Color***—Place 50 mL of Concentrated Glycerin in a Nessler tube and observe downward: the solution has no more color than the following control solution.

Control solution—Pipet 0.40 mL of iron (III) chloride hexahydrate colorimetric stock solution into a Nessler tube and add water to make 50 mL.

(2) ***Acidity or alkalinity***—To 2 mL of Concentrated Glycerin, add 8 mL of water and mix: the solution is neutral.

(3) ***Chloride***—Take 10.0 g of Concentrated Glycerin and perform the test. Prepare the control solution with 0.30 mL of 10 mmol/L hydrochloric acid VS (not more than 0.001 %).

(4) ***Sulfate***—Take 10.0 g of Concentrated Glycerin and perform the test. Prepare the control solution with 0.40 mL of 5 mmol/L sulfuric acid VS (not more than 0.002 %).

(5) ***Ammonium***—Take 5 mL of Concentrated Glycerin, add 5 mL of a solution of sodium hydroxide (1 in 10) and boil: the gas evolved does not change moistened red litmus paper to blue.

(6) ***Heavy metals***—Proceed with 5.0 g of Concentrated Glycerin according to Method 1 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(7) ***Calcium***—Take 5 mL of the solution obtained in (2), add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) ***Arsenic***—Prepare the test solution with 1.0 g of Concentrated Glycerin according to Method 1 and perform the test (not more than 2 ppm).

(9) ***Acrolein, glucose or other reducing substances***—Take 1.0 g of Concentrated Glycerin, add 1 mL of ammonia TS, mix and warm in a water-bath at 60 ºC for 5 minutes: no yellow color is produced. Take the solution out of the water-bath, add 3 drops of silver nitrate TS immediately and allow to stand in a dark place for 5 minutes: the color of the solution does not change and no turbidity is observed.

(10) ***Ethylene glycol and diethylene glycol***—Weigh accurately a suitable amount of Concentrated Glycerin and the internal standard, dissolve in methanol to make a solution containing 50 mg of Glycerin and 0.10 mg of the internal standard in 1 mL, and use

this solution as the test solution. Separately, weigh a suitable amount of Glycerin RS, Ethylene Glycol RS, Diethylene Glycol RS and the internal standard, dissolve in methanol to make a solution containing 2.0 mg, 0.050 mg, 0.050 mg and 0.10 mg in 1 mL of each, respectively, and use this solution as the standard solution. Perform the test with 1.0 μL of each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and determine each peak area by the automatic integration method: the ratio of the peak area of diethylene glycol from the test solution to that of the internal standard is not greater than the ratio of the peak area of diethylene glycol from the standard solution to that of the internal standard (0.10 %), and the ratio of the peak area of ethylene glycol from the test solution to that of the internal standard is not greater than the ratio of the peak area of ethylene glycol from the standard solution to that of the internal standard (0.10 %).

Internal standard—2,2,2-trichloroethanol

Operating conditions

Detector: A hydrogen flame ionization detector

Column: Coat the inside wall of a quartz tube, about 0.53 mm in internal diameter and about 30 m in length, to 3.0 μm thickness with cyanopropylphenyl- and 94 % dimethylpolysiloxan for gas chromatography.

Column temperature: Start at 100 °C, keep for 4 minutes, increase to 120 °C at the rate of 50 °C per minute and keep for 10 minutes, increase again to 220 °C at the rate of 50 °C per minute and keep at 220 °C for 6 minutes.

Injection port temperature: A constant temperature of about 220 °C

Detector temperature: A constant temperature of about 250 °C

Carrier gas: Helium

Flow rate: 4.5 mL/minute

Split ratio: About 1:10

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the resolution between the peaks of diethylene glycol and glycerin is not less than 1.5. The relative retention times of ethylene glycol, 2,2,2-trichloroethanol (95), diethylene glycol and glycerin are 0.3, 0.6, 0.8 and 1.0, respectively.

(11) ***Related substances***—Weigh accurately a suitable amount of Concentrated Glycerin, dissolve in water to make a solution containing 50 mg of glycerin in 1 mL, and use this solution as the test solution. Perform the test with 0.5 μL of the test solution as directed under Gas Chromatography under the following operating conditions and determine the areas of the peaks from the test solution: the content of each related substance is not more than 0.1 % and the total content of the related substances is not more than 1.0 %.

$$\text{Amount (\%) of related substances} = \frac{A_i}{A_S} \times 100$$

A_i: Peak area of each related substance from the test solution (excluding the solvent and diethyl glycol peak)

A_S: Total area of all peaks from the test solution

Operating conditions

Detector: A hydrogen flame ionization detector

Column: Coat the inside wall of a quartz tube, 0.53 mm in internal diameter and 30 m in length, to 3.0 μm thickness with 6 % cyanopropylphenyl- and 94 % dimethylpolysiloxan for gas chromatography

Column temperature: Maintain a constant temperature of about 100 °C until pouring, increase to 220 °C at the rate of 7.5 °C per minute and keep for 4 minutes.

Injection port temperature: A constant temperature of about 220 ºC

Detector temperature: A constant temperature of about 250 ºC

Carrier gas: Helium

Flow rate: 38cm/sec

Split ratio: About 1 : 10

System suitability

System performance: Weigh accurately a suitable amount of Diethylene Glycol RS and Glycerin RS, add water to make a solution containing 0.5 mg of each in 1 mL and use this solution as the system suitability solution. When the procedure is run with 0.5 μL of this solution under the above operating conditions, the resolution between the peaks of diethylene glycol and glycerin is not less than 7.0.

(12) ***Chloride***—Weigh accurately about 5 g of glycerin, transfer to a dried 100 mL round bottom flask, add 15 mL of morpholine, connect the flask to a reflux condenser and reflux gently for 3 hours. Wash the condenser with 10 mL of water, add the washing to the flask and carefully acidify with nitric acid. Transfer this solution to a colorimetric tube, add 0.50 mL of silver nitrate TS, add water to make 50 mL and use this solution as the test solution. Separately, take 0.20 mL of 0.020 mol/L hydrochloric acid, prepare in the same manner as the test solution, omitting the reflux procedure, and use this solution as the control solution. The turbidity of the test solution is not more intense than the control solution (not more than 0.003 %).

(13) ***Fatty acids and esters***—Mix 50 g of Concentrated Glycerin with 50 mL of freshly boiled and cooled water, add 10 mL of 0.1 mol/L sodium hydroxide VS, accurately measured, boil the mixture for 15 minutes, cool and titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: not more than 3.0 mL of 0.1 mol/L sodium hydroxide VS is consumed (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

(14) ***Readily carbonizable substances***—Take 5 mL

of Concentrated Glycerin, add carefully 5 mL of sulfuric acid, mix gently at a temperature between 18 ºC and 20 ºC and allow to stand for 1 hour at room temperature: the solution has no more color than Matching Fluid H.

Water Not more than 2.0 % (6 g, volumetric titration, direct titration)

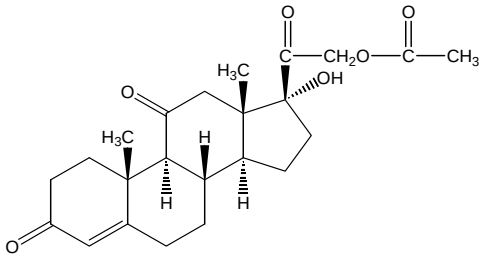
Residue on Ignition Weigh accurately about 10 g of Concentrated Glycerin in a tared crucible, heat to boiling and fire to burn immediately. Cool, moisten the residue with 1 to 2 drops of sulfuric acid and ignite cautiously to constant weight: the weight of the residue is not more than 0.01 %.

Assay Weigh accurately about 0.2 g of Concentrated Glycerin, transfer to an Erlenmeyer flask, add 50 mL of water and mix. Add exactly 50 mL of sodium periodate TS, shake and allow to stand at room temperature in a dark place for about 30 minutes. To this solution, add 10 mL of a mixture of water and ethylene glycol (1:1) and allow to stand for about 20 minutes. Add 100 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthanlein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.209 mg of $C_3H_8O_3$

Containers and Storage ***Containers***—Tight containers.

Cortisone Acetate



$C_{23}H_{30}O_6$: 402.48

[2-[(8*S*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Hydroxy-10,13-dimethyl-3,11-dioxo-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] acetate [*50-04-4*]

Cortisone Acetate, when dried, contains not less than 97.0 % and not more than 102.0 % of cortisone acetate ($C_{23}H_{30}O_6$).

Description Cortisone Acetate appears as white crystals or crystalline powder and is odorless.
Cortisone Acetate is sparingly soluble in methanol, slightly soluble in ethanol (99.5) and practically insoluble in water.

Melting point—About 240 °C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Cortisone Acetate and allow to stand for a while: a yellowish green color is produced and it gradually changes to orange-yellow. Examine the solution under ultraviolet light: the solution shows a pale green fluorescence. Add carefully 10 mL of water to this solution: the orange-yellow color of the solution is discharged and the solution remains clear.

(2) Determine the absorption spectra of solutions of Cortisone Acetate and Cortisone Acetate RS in methanol (1 in 50000) as directed under Ultraviolet-visible absorption Photometer: both spectra exhibit similar intensities of absorption at the same wavelengths..

(3) Determine the infrared spectra of Cortisone Acetate and Cortisone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Cortisone Acetate and Cortisone Acetate RS in acetone, respectively, evaporate the solutions to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +207 ~ +216° (after drying, 0.1 g, 10 mL of methanol, 100 mm).

Purity ***Related substances***—Dissolve 25 mg of Cortisone Acetate in 10 mL of a mixture of acetonitrile, water and acetic acid (100) (70 : 30 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of acetonitrile, water and acetic acid (100) (70 : 30 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with each 15 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions. And determine each peak area according to the automatic integration method: the peak area other than cortisone acetate obtained from the test solution is not greater than 1/2 times the peak area of cortisone acetate obtained from the standard solution. The sum of peak area other than the peak area of cortisone acetate obtained from the test solution is not greater than 1.5 times peak area of cortisone acetate obtained from the standard solution.

Operating conditions

Detector: An ultraviolet-visible Photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A : A mixture of water and acetonitrile (7 : 3)

Mobile phase B : B mixture of acetonitrile and water (7 : 3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-5	90	10
5-25	90→10	10→90
25-30	10	90

Flow rate: 1 mL/minute

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile, water and acetic acid (100) (70 : 30 : 1) to make exactly 10 mL. Confirm that the peak area of cortisone acetate obtained with 15 μL of this solution is equivalent to 8 to 12 % of that with 15 μL of the standard solution

System performance: When the procedure is run with 15 μL of the test solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cortisone acetate are not less than 10000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 3 times with 15μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cortisone acetate is not more than 5.0 %.

Time span of measurement: About 3 times as long as the retention time of cortisone acetate beginning after the solvent peak.

Loss on Drying Not more than 1.0 % (0.5 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 10 mg each of Cortisone Acetate and Cortisone Acetate RS, previously dried and accurately weighed, in 50 mL of methanol, add exactly 5 mL each of the internal standard solution, then add methanol to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Cortisone Acetate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of cortisone acetate } (C_{23}H_{30}O_6) = \text{Amount (mg) of Cortisone Acetate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and acetonitrile (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of Cortisone Acetate is about 12 minutes.

System suitability

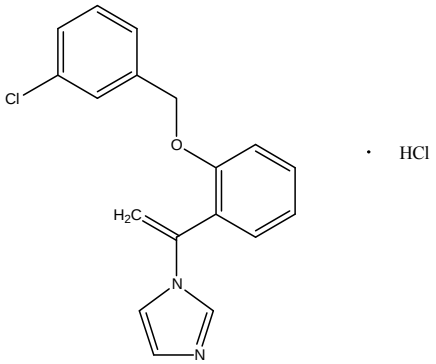
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cortisone acetate and internal standard sabstance are eluted in this order with the resolution between their peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of cortisone acetate to that of the internal standard is not more than 1.0 %

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Croconazole Hydrochloride



$C_{18}H_{15}ClN_2O \cdot HCl$: 347.24

1-[1-[2-[(3-Chlorophenyl)methoxy]phenyl]ethenyl] imidazole hydrochloride [*77174-66-4*]

Croconazole Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of croconazole hydrochloride ($C_{18}H_{15}ClN_2O \cdot HCl$).

Description Croconazole Hydrochloride appears as white to pale yellowish white crystals or cryatalline powder.

Croconazole Hydrochloride is very soluble in water, freely soluble in methanol, in acetic acid (100) or in acetone, practically insoluble in ether.

Identification (1) Determine the absorption spectra of solutions of Croconazole Hydrochloride and Croconazole Hydrochloride RS in methanol (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Croconazole Hydrochloride and Croconazole Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve about 50 mg of Croconazole Hydrochloride in 10 mL of water, add 2 mL of sodium hydroxide TS and 20 mL of ether, and shake. Wash the separated aqueous layer with two 10 mL portions of ether, and acidify the solution with 2 mL of dilute nitric acid: the solution responds to the qualitative tests for chloride.

Melting Point 148 ~ 153 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Croconazole Hydrochloride according to the method 4, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 50 mg of Croconazole Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and ammonia solution (28) (30 : 15 : 5 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot of the starting point from the test solution are not more than intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1.0 g, 60 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1.0 g).

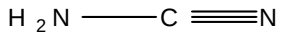
Assay Weigh accurately about 0.6 g of Croconazole Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS [indicator: 1 to 2 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)] until the color of this solution changes from blue-green through green to yellow-green. Perform a blank determination, and make any necessary correction.

1 mL of 0.1 mol/L perchloric acid
= 34.724 mg of $C_{18}H_{15}ClN_2O \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cyanamide



Aminonitrile CH_2N_2: 42.04

Cyanamide [*420-04-2*]

Cyanamide contains not less than 97.0 % and not more than 101.0 % of cyanamide (CH_2N_2), calculated on the anhydrous basis.

Description Cyanamide appears as white crystals or crystalline powder and has a faint, characteristic odor.
Cyanamide is very soluble in water, in methanol or in ethanol (95) and freely soluble in ether.
Cyanamide is hygroscopic.

pH—The pH of a solution of 1.0 g of Cyanamide in 100 mL of water is between 5.0 and 6.5.

Melting point—About 46 °C.

Identification (1) To 1 mL of a solution of Cyanamide (1 in 100), add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) Drop one or two drops each of the solutions of Cyanamide and Cyanimide RS in acetone (1 in 100) onto potassium bromide disks prepared as directed in the potassium bromide disk method under Infrared Sectrophotometry separately and air-dry the disks. Determine the infrared spectra of the disks as directed in the film method under Infrared Spectrometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Cyanamide in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Cyanamide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

Water Not more than 1.0 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

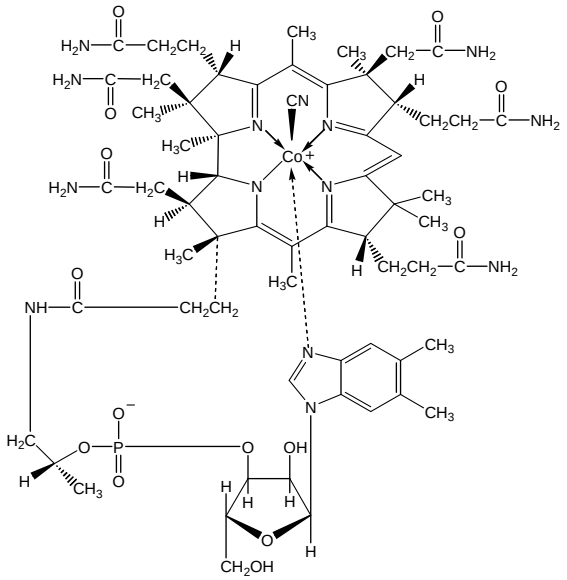
Assay Weigh accurately about 1 g of Cyanamide and dissolve in water to make exactly 250 mL. Pipet 15 mL of this solution, add 2 to 3 drops of dilute nitric acid, 10 mL of ammonia TS and exactly 50 mL of 0.1 mol/L silver nitrate VS and allow to stand for 15 minutes with occasional shaking. Add water to make exactly 100 mL, filter, discard the first 20 mL of the filtrate and pipet the subsequent exactly 50 mL. After neutralizing this solution with dilute nitric acid, add 3 mL of dilute nitric acid and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 2.1020 mg of CH_2N_2

Containers and Storage ***Containers***—Tight containers.

Storage—In a cold place.

Cyanocobalamin



Vitamin B_{12} $C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37

Cobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl][(2*S*)-1-[3-[(2*R*,3*R*,4*Z*,7*S*,9*Z*,12*S*,13*S*,14*Z*,17*S*,18*S*,19*R*)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1*H*-corrin-21-id-3-yl]propanoylamino]propan-2-yl]phosphate; cyanide [*68-19-9*]

Cyanocobalamin contains not less than 96.0 % and not more than 102.0 % of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), calculated on the dried basis.

Description Cyanocobalamin appears as dark red crystals or powder.
Cyanocobalamin is sparingly soluble in water and slightly soluble in ethanol (95).
Cyanocobalamin is hygroscopic.

Identification (1) Determine the absorption spectra of the test solution and the standard solution obtained in the Assay, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Cyanocobalamin with 50 mg of potassium bisulfate and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add drop-wise sodium hydroxide TS until a pale red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange color is immediately produced. Then add 0.5 mL of hydrochloric acid and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a distilling flask, dissolve in 5 mL of water and add 2.5 mL of hypophosphorous acid. Connect the flask with a short condenser and dip its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distill 1 mL into a test tube. To the test tube, add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 30 mg sodium fluoride and heat the contents to boil. Immediately add drop-wise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

pH Dissolve 0.10 g of Cyanocobalamin in 20 mL of freshly boiled and cooled water: the pH of this solution is between 4.2 and 7.0.

Purity (1) ***Clarity and color of solution***— Dissolve 20 mg of Cyanocobalamin in 10 mL of water: the solution is clear and red in color.

(2) ***Related substances***—Conduct this procedure using light-resistant vessels. Dissolve 10 mg of Cyanocobalamin in exactly 10 mL of the mobile phase, and use this solution as the test solution. Pipet 3 mL of the test solution, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the total area of the peaks other than cyanocobalamin from the test solution is not larger than the peak area of cyanocobalamin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 361 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: Dissolve 10 g of anhydrous disodium hydrogen phosphate in 1000 mL of water, and adjust the pH to 3.5 with phosphoric acid. To 147 mL of this solution add 53 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cyanocobalamin is about 7 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of this solution, and dissolve in the mobile phase to make exactly 10 mL. Confirm that the peak area of cyanocobalamin obtained from 20 μL of this solution is equivalent to 7 to 13 % of that of cyanocobalamin from the system suitability solution.

System performance: Perform this procedure quickly after the solution is prepared. To 25 mg of Cyanocobalamin add 10 mL of water, dissolve by warming if necessary, cool, add 0.5 mL of sodium toluenesulfonchloramide TS and 0.5 mL of 0.05 mol/L hydrochloric acid TS, add water to make 25 mL, shake, and allow to stand for 5 minutes. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, two principal peaks appear with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of cyanocobalamin is not more than 3.0 %.

Time span of measurement: About 4 times as long as the retention time of cyanocobalamin beginning after the solvent peak.

Loss on Drying Not more than 12 % (50 mg, at a pressure not exceeding 0.67 kPa, P_2O_5, 100 °C, 4 hours).

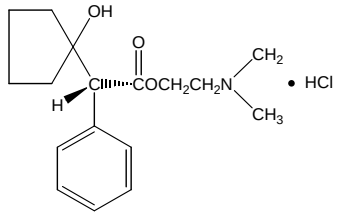
Assay Weigh accurately about 20 mg each of Cyanocobalamin and Cyanocobalamin RS (previously determine the loss on drying in the same manner as Cyanocobalamin), dissolve in water to make exactly 1000 mL, respectively, and use these solutions as the test solution and the standard solution. Determine the absorbances, A_T and A_S, of these solutions, respectively, at 361 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of cyanocobalamin } (C_{63}H_{88}CoN_{14}O_{14}P) = \text{Amount (mg) of Cyanocobalamin RS, calculated on the dried basis} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Cyclopentolate Hydrochloride



and enantiomer

$C_{17}H_{25}NO_3 \cdot HCl$: 327.85

2-(Dimethylamino)ethyl (1-hydroxycyclopentyl)(phenyl)acetate hydrochloride [*5870-29-1*]

Cyclopentolate Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of cyclopentolate hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$).

Description Cyclopentolate Hydrochloride is a white, crystalline powder and is odorless or has a characteristic odor.

Cyclopentolate Hydrochloride is very soluble in water, freely soluble in ethanol (95), in acetic acid (100) or in chloroform, sparingly soluble in acetic anhydride and practically insoluble in ether.

Identification (1) To 1 mL of a solution of Cyclopentolate Hydrochloride (1 in 100), add 1 mL of Reinecke salt TS: a pale red precipitate is produced.

(2) Dissolve 0.2 g of Cyclopentolate Hydrochloride in 2 mL of water, add 2 mL of sodium hydroxide TS and boil for 1 minute. After cooling, add 2 drops of nitric acid: a phenylacetic acid-like odor is perceptible.

(3) Determine the infrared spectra of Cyclopentolate Hydrochloride and Cyclopentolate Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Cyclopentolate Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 0.20 g of Cyclopentolate Hydrochloride in 20 ml of water: the pH of this solution is between 4.5 and 5.5.

Melting Point 135~ 138 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cyclopentolate Hydrochloride in 10 ml of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of

Cyclopentolate Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.20 g of Cyclopentolate Hydrochloride in 10 ml of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, n-butyl acetate, water and ammonia solution (28) (100 : 60 : 23 : 17) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of sulfuric acid in ethanol (99.5) (1 in 10) on the plate and heat at 120 °C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.05 % (1 g).

Assay Weigh accurately about 0.5 g of Cyclopentolate Hydrochloride, previously dried and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4 : 1) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.79 mg of $C_{17}H_{25}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Cyclophosphamide for Injection

Cyclophosphamide for Injection is a preparation for injection which is dissolved before use and Cyclophosphamide for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$: 261.09).

Method of Preparation Prepare as directed under Injections, with Cyclophosphamide Hydrate and Sodium Chloride.

Description Cyclophosphamide for Injection is a white powder.

Identification (1) Determine the infrared spectra of Cyclophosphamide for Injection and Cyclophosphamide Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both exhibit the similar intensities of absoption at the same wavenumbers.

(2) The retention time of the major peak in the chromatogram of the test solution under the Assay corresponds to that of the standard solution under the Assay.

pH Weigh accurately a portion of Cyclophosphamide for Injection, equivalent to about 0.2 g of anhydrous Cyclophosphamide and dissolve in 10 mL of water: pH of the solution is between 3.0 and 9.0 (30 minutes after preparation).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.20 EU/mg of cyclophosphamide.

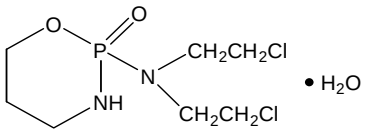
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 10 Cyclophosphamide for Injections. Weigh accurately a portion of Cyclophosphamide for Injection, equivalent to 0.10 g of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) and proceed as directed in the Assay under Cyclophosphamide Tablets.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Not exceeding 30 °C. Not exceeding 25 °C is recommended.

Cyclophosphamide Hydrate



$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$: 279.10

2-[*N*,*N*-Bis(2-chloroethyl)]amino-2-oxo-1,3,2λ^5-oxazaphosphinane hydrate [*6055-19-2*]

Cyclophosphamide Hydrate contains not less than 97.0 % and not more than 101.0 % of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P \cdot H_2O$).

Description Cyclophosphamide Hydrate is a white crystalline powder and is odorless.
Cyclophosphamide Hydrate is very soluble in acetic

acid (100), freely soluble in ethanol (95), acetic anhydride or in chloroform and soluble in water or in ether.

Melting point—45 ~ 53 °C.

Identification (1) Dissolve 0.1 g of Cyclophosphamide Hydrate in 10 mL of water and add 5 mL of silver nitrate TS: no precipitate is produced. Then boil this solution: a white precipitate is produced. Collect the precipitate and add dilute nitric acid to a portion of this precipitate: it does not dissolve. Add excess ammonia TS to another portion of the precipitate: it dissolves.

(2) Add 1 mL of diluted sulfuric acid (1 in 25) to 20 mg of Cyclophosphamide Hydrate and heat until white fumes are evolved. After cooling, add 5 mL of water and shake. Neutralize with ammonia TS, then acidify with dilute nitric acid: this solution responds to the Qualitative Tests (2) for phosphate.

(3) Determine the infrared spectra of Cyclophosphamide Hydrate and Cyclophosphamide Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***— Dissolve 0.20 g of Cyclophosphamide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 0.40 g of Cyclophosphamide Hydrate at a temperature not exceeding 20°C. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Cyclophosphamide Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water 5.5 ~ 7.0 % (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Cyclophosphamide Hydrate, add 15 mL of hydrogen chloride-ethanol TS and heat in a water-bath under a reflux condenser for 3.5 hours while protecting from moisture. Distill the ethanol under reduced pressure. Dissolve the residue in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid-1,4-dioxane VS (indicator: 2 drops of methylrosaniline chloride TS) until the color of the solution changes from blue through green to yellow. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 13.955 mg of $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Cyclophosphamide Tablets

Cyclophosphamide Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$: 261.09).

Method of Preparation Prepare as directed under Tablets, with Cyclophosphamide Hydrate.

Identification (1) Weigh a quantity of powdered Cyclophosphamide Tablets, equivalent to 50 mg of Cyclophosphamide, extract with 25 mL of chloroform, then filter. To 2 mL of the filtrate, add 0.5 g of potassium bromide, mix, evaporate chloroform, carefully removing the last trace of solvent in a small vacuum flask and use the residue to prepare a potassium bromide disk. Determine the infrared spectra of residue and Cyclophosphamide Hydrate RS (previously dried) as directed in the potassium bromide disk method under the infrared Spectrophotometry and compare the spectra at wavenumbers between 700 cm^{-1} and 1600 cm^{-1}: both spectra exhibit the same intensities of absorption at the same wavenumbers.

(2) The retention time of the major peak in the chromatogram of the test solution under the Assay corresponds to that of the standard solution under the Assay.

Dissolution Test Perform the test with 1 tablet of Cyclophosphamide Tablets at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of degassed water as the dissolution solution. Take not less than 20 mL of the dissolved solution 45 minutes after the start of the test, and filter through a membrane filter with a pore size of 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately a suitable amount of Cyclophosphamide RS, dissolve in water to make the same concentration as the test solution, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cyclophosphamide. The dissolution rate of Cyclophosphamide Tablets in 45 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) in 1 tablet

Operating conditions

Proceed as directed in the Assay.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 5 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cyclophosphamide is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Place 1 Tablet in a volumetric flask of suitable size so that the final concentration is about 500 μg/mL. Fill the flask about two-thirds full of water, shake until the Tablet is completely disintegrated, and dilute with water to volume. Filter, discard the first 10 mL of the filtrate, and use the remaining filtrate as the test solution. Separately, dissolve an accurately weighed quantity of Cyclophosphamide Hydrate RS in water, and dilute with water to obtain a solution having a known concentration of about 500 μg/mL. Use this solution as the standard solution. Place in separate test tubes 2.0 mL of the test solution, 2.0 mL of water (a blank), and 2.0 mL of the standard solution. Treat each tube as follows. Add 0.7 mL of perchloric acid solution, mix, and heat at 95 °C for 10 minutes. Cool, add 1.0 mL of sodium acetate TS, mix, add 1.6 mL of 4-(p-nitrobenzyl)-pyridine solution, mix, and heat at 95 °C for 10 minutes. Cool, add 8.0 mL of sodium hydroxide solution, and mix. Within 4 minutes, determine the absorbances of the solutions, as directed under Ultraviolet-visible Spectrophotometry, at the wavelength of maximum absorbance at 560 nm, against the blank. Determine the absorbances of the test solution and the standard solution A_T and A_s, respectively.

$$\text{Amount (mg) of cyclophosphamide } (C_7H_{15}Cl_2N_2O_2P\text{: } 261.09) \text{ in the Tablet} = \frac{T}{500} \times C \times \frac{A_T}{A_S}$$

T: Labeled quanity (mg) of anhydrous cyclophosphamide in the Tablet

C: Concentration (μg/mL) of cyclophosphamide corrected based on Cyclophosphamide Hydrate RS

Assay Weigh accurately and powder not less than 20 Cyclophosphamide Tablets. Weigh accurately a portion of the powder, equivalent to 0.1 g of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$), add 50 mL of water, shake, mix for 30 minutes and add water to make exactly 100 mL. Filter through a membrane filter, discard the first 40 or 50 mL of the filtrate, pipet 25.0 mL of the filtrate, add 5.0 mL of internal standard solution and add water to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately 25 mg of Cyclophosphamide Hydrate RS (previously determine the water content), dissolve in 25 mL of water, add 5.0 mL of internal standard solution and water to make exactly 50 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of cyclophosphamide to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of cyclophosphamide } (C_7H_{15}Cl_2N_2O_2P) = \text{Amount (mg) of anhydrous cyclophosphamide in Cyclophosphamide Hydrate RS} \times \frac{Q_T}{Q_S} \times 4$$

Internal standard solution—Weigh 0.185 g of *p*-oxyethylbenzoate and dissolve in 250 mL of ethanol (95) and add water to make exactly 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 195 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (70 : 30).

Flow rate: 1.5 mL/minute.

System suitability

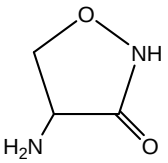
System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, cyclophosphamide and internal standard are eluted in this order with a resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cyclophosphamide to that of internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Cycloserine



$C_3H_6N_2O_2$: 102.09

(4*R*)-4-Amino-1,2-oxazolidin-3-one [*68-41-7*]

Cycloserine contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of cycloserine ($C_3H_6N_2O_2$: 102.09), calculated on the dried basis.

Description Cycloserine appears as white to pale yellowish white crystals or crystalline powder.
Cycloserine is soluble in water and sparingly soluble in ethanol (95).

Identification Determine the infrared spectra of Cycloserine and Cycloserine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +108 ~ +114° (2.5 g calculated on the dried basis, 2 mol/L sodium hydroxide TS, 50 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.5 g (potency) of Cycloserine in 10 mL of water is between 5.0 and 7.4.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Cycloserine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Condensation products***—Dissolve 20 mg of Cycloserine in sodium hydroxide TS to make exactly 50 mL. Determine the absorbance of this solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry: not more than 0.8.

Loss on Drying Not more than 1.5 % (0.5 g, in vacuum, 60 °C, 3 hours)

Residue on Ignition 0.5 % (1 g)

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be between 6.0 and 6.1.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 40 mg (potency) of Cycloserine, dissolve in sterile purified water to make a solution so that each mL contains 400 μg (potency), pipet a sutiable amount of this solution, add 1 % phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100.0 μg (potency) and 50.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution. Separately, weigh accurately about 40 mg (potency) of Cycloserine RS, dissolve in sterile purified water so that each mL contains 400 μg (potency), and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C and use within 24 hours. Pipet a suitable amount of the standard stock solution, add 1 % phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100.0 μg (potency) and 50.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Cycloserine Capsules

Cycloserine Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of cycloserine ($C_3H_6N_2O_2$: 102.09).

Method of Preparation Prepare as directed under Capsules, with Cycloserine.

Identification (1) To an amount of Cycloserine Capsules, equivalent to 10 mg (potency) of cycloserine, add 2 mL of water, shake, and filter. To the filtrate add 1 to 2 drops of iron (III) chloride: a dark red color develops.

(2) Dissolve an amount of Cycloserine Capsules, equivalent to 5 mg (potency) of cycloserine, in 10 mL of a solution of sodium hydroxide (1 in 250). To 1 mL of this solution add 3 mL of dilute acetic acid, shake, and add 1 mL of cycloserine reagent TS: a blue color develops.

Loss on Drying Not more than 1.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours)

Dissolution Test Perform the test with 1 capsule of Cycloserine Capsules at 100 revolutions per minute according to Method 1 under Dissolution Test, using 900 mL of pH 6.8 phosphate buffer solution as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately a suitable amount of Cycloserine RS, dissolve in pH 6.8 phosphate buffer solution to make a solution so that each mL contains about 0.25 mg (potency), and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cycloserine. The dissolution rate of Cycloserine Capsules in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of cycloserine ($C_3H_6N_2O_2$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of cycloserine ($C_3H_6N_2O_2$) in 1 capsule

pH 6.8 phosphate buffer solution—To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 22.4 mL of 0.2 mol/L sodium hydroxide and water to make 200 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 219 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 0.5 g of sodium 1-decanesulfonate in 800 mL of water, add 50 mL of acetonitrile and 5 mL of acetic acid (100), mix well, adjust the pH to 4.4 with 1 mol/L sodium hydroxide, filter, and degas.

Flow rate: 1 mL/minute

Column temperature: A constant temperature of about 30 °C

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor is not more than 1.8.

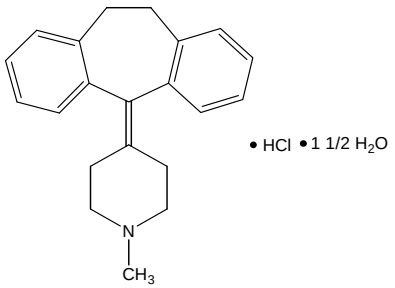
System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cycloserine is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Cycloserine. Weigh accurately the contents of not less than 20 Cycloserine Capsules. Weigh accurately an amount of the contents, equivalent to about 40 mg potency) according to the labeled potency, add sterile purified water to make exactly 100 mL, shake vigorously, and filter if necessary. Pipet a suitable amount of this solution, add 1 % phosphate buffer solution (pH 6.0) to make the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Cyproheptadine Hydrochloride Hydrate



$C_{21}H_{21}N \cdot HCl \cdot 1^1/_2\, H_2O$: 350.88

4-(5H-Dibenzo[a,d]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate [*41354-29-4*]

Cyproheptadine Hydrochloride Hydrate, when dried, contains not less than 98.5 % and not more than 101.0 % of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$: 323.86).

Description Cyproheptadine Hydrochloride Hydrate is a white to pale yellow, crystalline powder, is odorless and has a slightly bitter taste.

Cyproheptadine Hydrochloride Hydrate is freely soluble in methanol or in acetic acid (100), soluble in chloroform, sparingly soluble in ethanol (95), slightly soluble in water and practically insoluble in ether.

Identification (1) Dissolve 0.1 g of Cyproheptadine Hydrochloride Hydrate in 10 mL of methanol, apply 1 drop of this solution on filter paper, air-dry and examine under ultraviolet light (main wavelength: 254 nm): the solution shows a pale blue fluorescence.

(2) Weigh 0.1 g of Cyproheptadine Hydrochloride Hydrate, transfer to a separatory funnel, dissolve in 5 mL of chloroform, add 4 mL of water and 1 mL of sodium carbonate TS and shake. Transfer the chloroform layer to another separatory funnel and wash with 4 mL of water by shaking well. Filter the chloroform layer through absorbent cotton moistened previously with chloroform and evaporate the filtrate to dryness. Dissolve the residue in 8 mL of dilute ethanol by warming at 65 °C. Rub the inner wall of the container with a glass rod while cooling until crystallization begins and allow to stand for 30 minutes. Filter and collect the crystals and dry at 80 °C for 2 hours: the crystals melt between 111 °C and 115 °C.

(3) Determine absorption spectra of the solutions of Cyproheptadine Hydrochloride Hydrate and Cyproheptadine Hydrochloride Hydrate RS, in ethanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A saturated solution of Cyproheptadine Hydrochloride Hydrate responds to the Qualitative Tests (2)

for chloride.

Purity (1) ***Acid***—Dissolve 2.0 g of Cyproheptadine Hydrochloride Hydrate in 25 mL of methanol and add 1 drop of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color is observed.

(2) ***Heavy metals***— Proceed with 1.0 g of Cyproheptadine Hydrochloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying 7.0 ~ 9.0 % (1g, in vacuum at the pressure not exceeding 0.67 kPa, 100 °C, 5 hours).

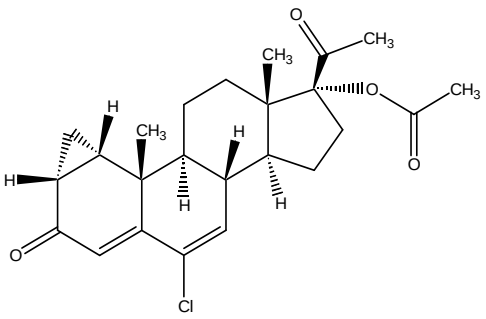
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Cyproheptadine Hydrochloride Hydrate, previously dried, and dissolve in 20 ml of acetic acid (100) by warming at 50 °C. After cooling, add 40 ml of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid VS
= 32.386 mg of $C_{21}H_{21}N \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Cyproterone Acetate



$C_{24}H_{29}ClO_4$: 416.94

17-Acetyloxy-6-chloro-1α,2α-methylenepregna-4,6-diene-3,20-dione [*427-51-0*]

Cyproterone Acetate contains not less than 97.0 % and not more than 103.0 % of cyproterone acetate ($C_{24}H_{29}ClO_4$), calculated on a dried basis.

Description Cyproterone Acetate is a white, crystalline powder.
Cyproterone Acetate is very soluble in methylene chloride, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water

Identification (1) To about 1 mg of Cyproterone Acetate, add 2 mLof sulphuric acid and heat on a water-bath for 2 min, and cool. Add the solution cautiously to 4 mL of water and shake. The solution becomes violet.

(2) Determine the infrared spectra of Cyproterone Acetate and Cyproterone Acetate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 20 mg of Cyproterone Acetate in methylene chloride to make 10 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Cyproterone Acetate RS in methylene chloride to make 5 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of cyclohexane and ethyl acetate (50:50) to a distance of about 15 cm, and dry the plate in air. Examine in ultraviolet light at 254 nm, and the principal spot from the test solution and that from the standard solution show the same R_f value.

(4) Incinerate about 30 mg of Cyproterone Acetate with 0.3 g of anhydrous sodium carbonate over a naked flame for about 10 minutes. Cool, dissolve the residue in 5 mL of dilute nitric acid, and filter. To 1 mL of the filtrate, add 1 mL of water. The solution responds to the Qualitative Tests (2) for chloride.

(5) In a test tube (about 180 mm × 18 mm), place about 15 mg of Cyproterone Acetate and 0.15 mL of phosphoric acid. Close the tube with a stopper through which passes a small test tube (about 100 mm × 10 mm) containing water to act as a condenser. On the outside of the smaller tube, hang a drop of 5 % lanthanum nitrate solution. Place the apparatus in a water-bath for 5 minutes, then take out the smaller tube. Remove the drop and mix it with 0.05 mL of 0.01 mol/L iodine TS on a tile. Add at the edge 0.05 mL of 2 mol/L ammonia. After 1 to 2 minutes, a blue color develops at the junction of the two drops; the color intensifies and persists for a short time.

Specific Optical Rotation $[\alpha]_D^{20}$: +152 ~ +157° (0.25 g previously dried, acetone, 20 mL, 100 mm).

Purity ***Related substances***—Dissolve 10.0 mg of Cyproterone Acetate in acetonitrile to make exactly 10 mL, and use this solution as the test solution. Dilute 1.0 mL of the test solution to 100 mL with acetonitrile, and use this solution as the standard solution (1). Dissolve 5 mg of Medroxyprogesterone acetate RS in acetonitrile to make exactly 50 mL. Dilute 1.0 mL of the solution to 10 mL with the standard solution (1), and use this solution as the standard solution (2). Perform the

test with 20 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions. The sum of the areas of all the peaks, other than the principal peak, from the test solution is not greater than 0.5 times the area of the principal peak from the standard solution (1) (0.5 %). Disregard any peak with an area less than 0.05 times that of the principal peak from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (60:40**).**

Flow rate: 1.5 mL/min.

System suitability

System performance: Adjust the sensitivity of the system so that the height of the principal peak from the standard solution (1) is at least 50 % of the full scale of the recorder. When the procedure is run with the standard solution (2), the resolution between the peaks of cyproterone acetate and medroxyprogesterone acetate is not less than 3.0.

Loss on Drying Not more than 0.5 % (1 g, 80 °C, not exceeding 0.67 kPa, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

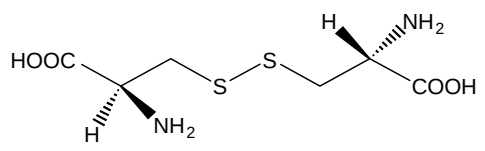
Assay Dissolve 50.0 mg of Cyproterone Acetate in methanol to make exactly 50 mL. Dilute 1.0 mL of the solution to 100 mL with methanol, and use this solution as the test solution. Using methanol as the blank solution, measure the absorbance (A) of the test solution at 282 nm.

$$\text{Amount (mg) of cyproterone acetate } (C_{24}H_{29}ClO_4) = \frac{A}{414} \times 50000$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

L-Cystine



$C_6H_{12}N_2O_4S_2$: 240.30

cystine [*56-89-3*]

L-Cystine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-cystine ($C_6H_{12}N_2O_4S_2$).

Description L-Cystine is a white crystalline powder.
L-Cystine is practically insoluble in water or in ethanol (95).
L-Cystine dissolves in dilute sodium hydroxide TS.

Identification (1) Determine the infrared spectra of L-Cystine and L-Cystine RS in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Examine the thin-layer chromatograms obtained in the test for ninhydrin-positive substances under the Purity (2): The principal spot from the test solution (2) is same in color and R_f value the principal spot from the standard solution (1).

(3) To 0.1 g of L-Cystine, add carefully 1 mL of strong hydrogen peroxide TS and 0.1 mL of iron (III) chloride TS and allow to cool. Add 1 mL of dilute hydrochloric acid TS, 5 mL of water and 1 mL of barium chloride TS: a turbidity or a white precipitate develops within 3 minutes.

Specific Optical Rotation $[\alpha]_D^{20}$: -218 ~ 224° (after drying, 0.5 g, 1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of L-Cystine in 10 mL of dilute hydrochloric acid TS: The solution is clear and not more intensely colored than Color Matching Solution F.

(2) ***Ninhydrin-positive substances***—Dissolve 0.1 g of L-Cystine in 10 mL of 1 mol/L hydrochloric acid VS and use this solution as the test solution (1). Pipet 1 mL of the test solution, add water to make 50 mL and use this solution as the test solution (2). Separately, dissolve 10 mg of L-Cystine RS in 1 mL of 1 mol/L hydrochloric acid VS, add water to make 50 mL and use this solution as the standard solution (1). To 2 mL of the test solution (2) add water to make 20 mL and use this solution as the standard solution (2). Dissolve 10 mg of L-Cystine RS and 10 mg of arginine hydrochloride in 1 mL of 1 mol/L hydrochloric acid VS, add water to make 25 mL and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution (1), the test solution (2), the standard solution (1), the standard solution (2) and the standard solution (3) on a plate of silica gel for thin-layer chromatography. Develop the plates with an upper layer of a mixture of ammonia solution (28) and 2-propanol (30 : 70) to a distance of about 15 cm and air-dry the plates. Spray evenly with a solution of ninhydrin VS on plate and heat at 100 °C to 150 °C for 15 minutes: the spots other than principal spot from the

test solution (1) are not more intense than the spot from the standard solution (2) and the chromatogram obtained from the standard solution (3) shows two clearly separated spots.

(3) ***Chloride***—Perform the test with 0.50 g of L-Cystine. Prepare the control solution with 0.29 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.020 %).

(4) ***Sulfate***—Dissolve 0.50 g of L-Cystine in 5 mL of dilute hydrochloric acid TS, add water to make 15 mL and use this solution as the test solution. Prepare the control solution with 0.32 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid TS and add water to make 15 mL (not more than 0.030 %).

(5) ***Ammonium***—Perform the test with 0.25 g of L-Cystine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.020 %).

(6) ***Heavy metals***—Proceed with 2.0 g of L-Cystine according to Method 1 under Heavy Metals Limit Test and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(7) ***Iron***—Dissolve 1.0 g of L-Cystine in 10 mL of dilute hydrochloric acid TS in a separator, extract with three quantities, each 10 mL of 4-methyl-2-pentanone. To the 4-methyl-2-pentanone layer add 10 mL of water and mix with shaking for 3 minutes. Take the water layer, add 30 mL of acetic acid-sodium acetate buffer solution, pH 4.5 and use this solution as the test solution. Perform the test with the test solution according to Method B. Prepare the control solution with 1.0 mL of standard iron solution (not more than 10 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105°C, constant mass)

Residue on Ignition Not more than 0.1 % (1 g).

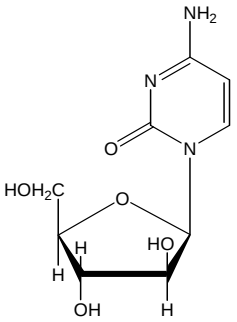
Assay Weigh accurately about 0.1 g of L-Cystine, previously dried, transfer to a capped flask and dissolve in 2 mL of dilute sodium hydroxide VS and 10 mL of water. Add 10 mL of potassium bromide solution (2 in 10), 50.0 mL of 1/60 mol/L potassium bromate VS and 15 mL of dilute hydrochloric acid TS. Stopper the flask and cool in iced water. Allow to stand in the dark for 10 minutes and add 1.5 g of potassium iodide. After 1 minute, titrate with 0.1 mol/L sodium thiosulfate (indicator: 2 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 1/60 mol/L solution of potassium bromate VS = 2.4030 mg of $C_6H_{12}N_2O_4S_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Cytarabine



$C_9H_{13}N_3O_5$: 243.22

4-Amino-1-[(2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one
[*147-94-4*]

Cytarabine, when dried, contains not less than 98.5 % and not more than 101.0 % of cytarabine ($C_9H_{13}N_3O_5$).

Description Cytarabine appears as white crystals or crystalline powder.
Cytarabine is freely soluble in water, soluble in acetic acid (100), very slightly soluble in ethanol (95) and practically insoluble in ether.

Melting point—About 214 °C (with decomposition)

Identification (1) Determine the absorption spectra of solutions of Cytarabine and Cytarabine RS in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Cytarabine and Cytarabine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +154 ~ +160° (after drying, 0.1 g, water, 10 mL, 100 mm)

pH Dissolve 0.20 g of Cytarabine in 20 mL of water: the pH of this solution is between 6.5 and 8.0.

Absorbance $E_{1cm}^{1\%}$ (282 nm): 530 ~ 570 (after drying, 2 mg, 0.1 mol/L hydrochloric acid TS, 200 mL)

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Cytarabine in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 1.0 g of Cytarabine. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009 %).

(3) ***Heavy metals***—Proceed with 1.0 g of

Cytarabine according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Cytarabine according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Cytarabine in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with 1-butanol saturated with water to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution. Spray evenly acidic potassium permanganate TS on the plate: any spot other than the principal spot does not appear.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Weigh accurately about 0.2 g of Cytarabine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Methods in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.161 mg of $C_9H_{13}N_3O_5$

Containers and Storage ***Containers***—Tight containers.

Cytarabine for Injection

Cytarabine for Injection is a preparation for injection which is dissolved before use. Cytarabine for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of cytarabine ($C_9H_{13}N_3O_5$: 243.22).

Method of Preparation Prepare as directed under Injections, with cytarabine.

Description Cytarabine for Injection appears as white powder.

Identification The retention time of the major peak in the chromatogram of the test solution under the Assay corresponds to that in the chromatogram of the standard solution under the Assay.

pH 4.0 ~ 6.0 (water, 20 mg/mL).

Water Not more than 3.0 % (1 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.07 EU/mg of cytarabine.

Uniformity of Dosage Units It meets the requirement.

Assay Take 10 Cytarabine for Injection, dissolve in water to make a solution containing 10 mg of cytarabine ($C_9H_{13}N_3O_5$) per mL. Pipet 5.0 mL of this solution and add water to make exactly 50 mL. Pipet 3.0 mL of this solution, add 5.0 mL of the internal standard solution and mobile phase to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately 50 mg of Cytarabine RS (previously dried at 60 °C, not more than 0.67 kPa, for 3 hours), dissolve in water to make exactly 50 mL. Pipet 3.0 mL of this solution and add 5.0 mL of the internal standard solutio and mobile phase to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Cytarabine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of cytarabine } (C_9H_{13}N_3O_5) = \text{Amount (mg) of Cytarabine RS} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—0.14 % *p*-toluenic acid in methanol.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, 4 mm to 4.6 mm in internal diameter and 25 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 0.69 g of sodium dihydrogen phosphate dihydrate and 1.34 g of sodium monophosphate in 950 mL of water. Add 50 mL of methanol and mix.

Flow rate: 1 mL/minute.

System suitability

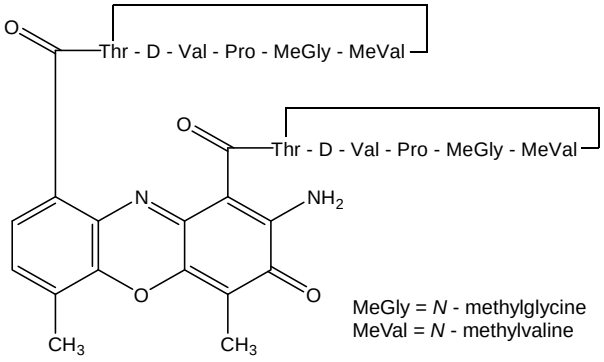
System performance: Weigh a sufficient portion of Uracylarabinoside RS and Cytarabine RS, dissolve in water to make a solution containing 10 μg and 600 μg per 1 mL, respectively, dilute with same volume of internal standard solution, mix with about four times

volume of mobile phase. When the procedure is run with 10 μL of this solution under the above operating condition, cytarabine, uracylarabinoside and *p*-toluenic acid are eluted in this order with a resolution between cytarabine and uracylarabinoside peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cytarabine to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Dactinomycin



$C_{62}H_{86}N_{12}O_{16}$: 1255.42

2-Amino-*N,N'-bis*[(6*S*,9*R*,10*S*,13*R*,18a*S*)-6,13-diisopropyl-2,5,9-trimethyl-1,4,7,11,14-pentaoxohexadecahydro-1*H*-pyrrolo[2,1-i][1,4,7,10,13]oxatetraazacyclohexadecin-10-yl]-4,6-dimethyl-3-oxo-3*H*-phenoxazine-1,9-dicarboxamide [*50-76-0*]

Dactinomycin is a peptide substance having antitumor activity produced by the growth of *Streptomyces parvulus*.
Dactinomycin, when dried, contains not less than 950 μg (potency) and not more than 1030 μg (potency) of dactinomycin ($C_{62}H_{86}N_{12}O_6$: 1255.42) per mg.

Description Dactinomycin appears as orange to red crystalline powder.
Dactinomycin is freely soluble in acetone, sparingly soluble in acetonitrile or in methanol, slightly soluble in ethanol (99.5) and practically insoluble in water.

Identification (1) Determine the absorption spectrum of the solution of Dactinomycin in methanol (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Dactinomycin and Dactinomycin RS in 10 mL of acetone and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and methanol (4:2:1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254nm): the principal spot obtained from the test solution shows the same R_f value as the spots obtained from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: -292 ~ -317° (10 mg after drying, methanol, 10 mL, 100 mm).

Loss on Drying Not more than 5.0 % (1 g, in vacuum, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Dactinomycin is used in a sterile preparation.

Bacterial Endotoxins Less than 100 per mg (potency) of dactinomycin, when Dactinomycin is used in a sterile preparation.

Assay Weigh accurately 60 mg (potency) each of Dactinomycin and Dactinomycin RS, previously dried, dissolve each in the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas of dactinomycin, A_T and A_S.

$$\text{Amount [mg (potency)] of dactinomycin } (C_{62}H_{86}N_{12}O_{16}) = \text{Amount [mg (potency)] of Dactinomycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: a mixture of 0.02 mol/L acetic acid, sodium acetate TS and acetonitrile (25:23)

Flow rate: Adjust the flow rate so that the retention time of dactinomycin is about 23 minutes.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates

of the peak of dactinomycin is not less than 2000 with the symmetry factor being not more than 1.5.

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dactinomycin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dactinomycin for Injection

Dactinomycin for Injection is a preparation for injection, which is dissolved before use.
Dactinomycin for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of dactinomycin ($C_{62}H_{86}N_{12}O_{16}$: 1255.42).

Method of Preparation Prepare as directed under Injections, with Dactinomycin.

Description Dactinomycin for Injection appears as yellow to light red powder.

Identification (1) Dilute Dactinomycin for Injection with methanol to make a solution containing 25 μg (potency) per mL, and perform the test as directed in Identification (1) under Dactinomycin.

(2) Perform the test as directed in Identification (2) under Dactinomycin.

pH Dissolve a portion of Dactinomycin for Injection, equivalent to 5.0 mg (potency) of dactinomycin, in 11 mL of water: the pH of the solution is between 5.5 and 7.5.

Loss on Drying Not more than 4.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 100 EU/mg (potency) of dactinomycin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh a portion of Dactinomycin for Injection, equivalent to 250 μg (potency), add exactly 10 mL of the mobile phase to render the concentration of 25 μg (potency) per mL and use this solution as the test solution. Separately, weigh accurately 250 μg (potency) of Dactinomycin RS, add exactly 10 mL of the mobile phase to render the concentration of 25 μg (potency) per mL and use this solution as the standard solution. Prepare the test solution and the standard solution under protection from light. Perform the test with 10 μL each of the test solution and the standard solution as directed in the Assay under Dactinomycin according to the following operating conditions.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 mm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel or ceramic for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and water (6:4)

Flow rate: 2.5 mL/minute

System suitability

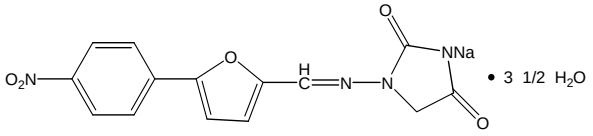
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of dactinomycin is not less than 1200 with the symmetry factor being not more than 2.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dactinomycin is not more than 3.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Dantrolene Sodium Hydrate



$C_{14}H_9N_4NaO_5 \cdot 3 \cdot 1/2\ H_2O$: 399.29

Sodium(E)-1-(((5-(4-nitrophenyl)furan-2-yl)methylene)amino)imidazolidine-2,4-dionehemiheptahydrate [*24868-20-0*]

Dantrolene Sodium contains not less than 98.0 % and not more than 101.0 % of dantrolene sodium ($C_{14}H_9N_4NaO_5$), calculated on the anhydrous basis.

Description Dantrolene Sodium Hydrate is a yellowish orange to deep orange, crystalline powder.
Dantrolene Sodium Hydrate is soluble in propylene glycol, sparingly soluble in methanol, slightly soluble in ethanol (95), very slightly soluble in water or in acetic acid (100) and practically insoluble in acetone, in

tetrahydrofuran or in ether.

Identification (1) Determine the absorption spectra of solutions of Dantrolene Sodium Hydrate and Dantrolene Sodium Hydrate RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dantrolene Sodium Hydrate and Dantrolene Sodium Hydrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both exhibit similar intensity of absorption at the same wavenumbers.

(3) To 0.1 g of Dantrolene Sodium Hydrate, add 20 mL of water and 2 drops of acetic acid (100), shake well and filter: the filtrate responds to the Qualitative Tests (1) for sodium salt.

Purity (1) ***Alkali***—To about 0.7 g of Dantrolene Sodium Hydrate, add 10 mL of water, shake well and centrifuge or filter through a membrane filter. To 5 mL of the clear supernatant liquid or the filtrate, add 45 mL of water, 3 drops of phenophthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS: a red color is not observed.

(2) ***Heavy metals***—Proceed with 1.0 g of Dantrolene Sodium Hydrate according to Method 2 and perform the test**.** Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 50.0 mg of Dantrolene Sodium Hydrate in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100), add ethanol (99.5) to make exactly 100 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add ethanol (99.5) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area from the test solution and the standard solution by the automatic integration method: the total area of all peaks other than the peak of dantrolene from the test solution is not larger than the peak area of dantrolene from the standard solution.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 300 nm).

Column : A stainless steel column, 4 mm in internal diameter and about 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature : A constant temperature of about 30 °C.

Mobile phase: A mixture of hexane, acetic acid (100) and ethanol (99.5) (90 : 10 : 9).

Flow rate : Adjust the flow rate so that the retention time of dantrolene is about 8 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dantrolene from 10 μL of the standard solution is 10 % to 40 % of the full scale.

System performance: Dissolve 5 mg of Dantrolene Sodium Hydrate and 0.1 g of theophylline in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100) and add ethanol (99.5) to make 100 mL. To 10 mL of this solution, add ethanol (99.5) to make 100 mL and use this solution as the standard solution. When the procedure is run with 10 μL of the standard solution under the above operating conditions, theophylline and dantrolene are eluted in this order with the resolution between their peaks being not less than 6.

Time span of measurement: About twice as long as the retention time of Dantrolene after the solvent peak.

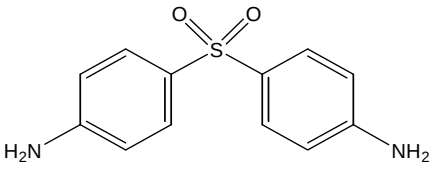
Water 14.5 ~ 17.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately 0.7 g of Dantrolene Sodium Hydrate, dissolve in 180 mL of a mixture of propylene glycol and acetone (1 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.624 mg of $C_{14}H_9N_4NaO_5$

Containers and Storage ***Containers***—Tight containers.

Dapsone



D.D.S $C_{12}H_{12}N_2O_2S$: 248.30

4-(4-Aminophenyl)sulfonylaniline [*80-08-0*]

Dapsone contains not less than 98.0 % and not more than 102.0 % of dapsone ($C_{12}H_{12}N_2O_2S$), calculated on the anhydrous basis.

Description Dapsone is a white or pale yellow crystalline powder.

Dapsone is freely soluble in acetone, slightly soluble in ethanol (95) and practically insoluble in water.

Dapsone dissolves in dilute inorganic acid,

Identification (1) Determine the absorption spectra

of solutions of Dapsone and Dapsone RS in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dry a suitable amount of Dapsone and Dapsone RS and measure the infrared spectra as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavenumbers.

Melting Point 175 ~ 181 °C.

Purity (1) ***Selenium***—Add 0.5 mL of a mixture containing perchloric acid and sulfuric acid (1 : 1), 2 mL of nitric acid to about 0.10 g of Dapsone and heat in a steam bath. Cool the solution until a brown gas is not evolved and the solution becomes clear with an evanescent yellowish color. Then, add 4 mL of nitric acid and water to make exactly 50 mL. Use this solution as the test solution. Separately, pipet 3 mL of selenium standard solution, add 0.5 mL of a mixture containing perchloric acid and sulfuric acid (1 : 1), 6 mL of nitric acid and water to make exactly 50 mL. Use this solution as the standard solution. Perform the test on the standard and the test solution as directed under the Atomic Absorption Spectrophotometry. Measure the absorbance A_T for the test solution and A_S for the standard solution when the reading of the display rapidly rises and, then, reaches a plateau. A_T is less than A_S (not more than 30 ppm). This test is performed using a hydride generator and a heating cell.

Lamp : Selenium hollow-cathode lamp
Wavelength : 196.0 nm
Atomizing temperature : When an electric furnace is used, the temperature is set at about 1000 °C.
Carrier gas: Nitrogen or argon

(2) ***Related substances***—Dissolve 0.50 g of Dapsone in 50.0 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of this solution accurately and add methanol to make exactly 100 mL and use this solution as the standard solution (1). Pipet 10.0 mL of the standard solution (1), add methanol to make exactly 50 mL and use this as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethylacetate, n-heptane, methanol and 13.5 mol/L of ammonia reagent mixture (20 : 20 : 6 : 1) and air-dry the plate. Spray evenly 0.1 % 4-dimethylaminocinnam aldehyde solution of ethanol (95) and hydrochloric acid mixture (99:1) on the plate: the spot having a strong intensity second to the principal spot from the test solution is not more intense than that from the standard solution (1). The spots other than the above two spots from the test solution are not more intense than that from the standard solution (2).

Loss on Drying Not more than 1.5 % (1 g, 105 ~105 °C, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg of Dapsone, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of the solution, add methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Dapsone RS, diluted similarly to the test solution with methanol and use this solution as the standard solution. Perform the test with 10 μL each of the test and standard solution as directed under Liquid Chromatography. Determine each peak area, A_T and A_S, of dapsone for the test solution and the standard solution.

$$\text{Amount (mg) of dapsone } (C_{12}H_{12}N_2O_2S) = \text{Amount (mg) of Dapsone RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with porous silica for liquid chromatography (10 μm in particle diameter).

Mobile phase: Mix 100 mL of 2-propanol, 100 mL of ethylacetate, 100 mL of acetonitrile and pentane to make 1000 mL. Allow the mixture to cool to room temperature.

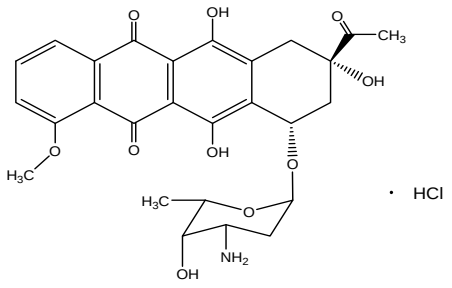
System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dapsone is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Daunorubicin Hydrochloride



$C_{27}H_{29}NO_{10}$·HCl: 563.98

(7*S*,9*S*)-9-Acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dionehydrochloride [*23541-50-6*]

Daunorubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces peucetius*.
Daunorubicin Hydrochloride contains not less than 940 μg (potency) and not more than 1050 μg (potency) per mg of daunoarubicin hydrochloride ($C_{27}H_{29}NO_{10}$·HCl), calculated on the dried basis.

Description Daunorubicin Hydrochloride appears as red powder.
Daunorubicin Hydrochloride is very soluble in water and in methanol and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of the solutions of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride RS in methanol (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: +250 ~ +275° (15 mg calculated on the dried basis, methanol, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.15 g of Daunorubicin Hydrochloride in 30 mL of water is between 4.5 and 6.0.

Absorbance $E_{1cm}^{1\%}$ (495 nm): 210 ~ 250 (10 mg calculated on the dried basis, methanol, 500 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 20 mg of Daunorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) ***Heavy metals***—Proceed with 1.0 g of Daunorubicin Hydrochloride according to Method 2 and perform the test. Prepare the control solution 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 10 mg of Daunorubicin Hydrochloride in 5 mL of methanol and use this solution as the test solution. Pipet 3 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water and acetic acid (100) (15:5:1:1) to a distance of about 10 cm and air-dry the plate. Examine the spots with the naked eye: the spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Loss on Dyring Not more than 7.5 % (0.1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Daunorubicin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 4.3 EU/mg (potency) of Daunorubicin Hydrochloride, when Daunorubicin Hydrochloride is used in a sterile preparation.

Histamine It meets the requirement, when Daunorubicin Hydrochloride is used in a sterile preparation,. Weigh appropriate amount of Daunorubicin Hydrochloride, dissolve in water, make the solution so that each mL contains 5.0 mg, and use the solution as the test solution.

Assay Weigh accurately an amount of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of daunorubicin to that of the internal standard..

$$\text{Amount [μg (potency)] of Daunorubicin hydrochloride } (C_{27}H_{29}NO_{10}\cdot HCl) = \text{Amount [μg (potency)] of Daunorubicin hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 2-naphthalenesulfonic acid hydrate in the mobile phase (1 in 100)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 300 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust the pH of a mixture of water and acetonitrile (31:19) to 2.2 with phosphoric acid

Flow rate: Adjust the flow rate so that the retention time of daunorubicin is about 9 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above op-

erating conditions, 2-naphthalenesulfonic acid hydrate and daunorubicin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the operating conditions, the relative standard deviation of the ratios of the peak area of daunorubicin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Daunorubicin Hydrochloride for Injection

Daunorubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.
Daunorubicin Hydrochloride for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of daunorubicin hydrochloride ($C_{27}H_{29}NO_{10}\cdot HCl$: 563.98).

Method of Preparation Prepare as directed under Injections, with Daunorubicin Hydrochloride.

Description Daunorubicin Hydrochloride for Injection appears as orange to red powder.

Identification Determine the retention times according to the Assay: the retention time of the principal peak from the test solution is the same as that from the standard solution.

pH Dissolve a portion of Daunorubicin Hydrochloride for Injection, equivalent to 50 mg (potency) of daunorubicin hydrochloride, in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

Loss on Drying Not more than 4.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 4.3 EU/mg (potency) of daunorubicin hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

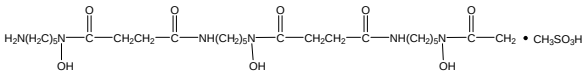
Uniformity of Dosage Units It meets the requirement.

Histamine It meets the requirement, when the test is performed as directed in Histamine under Daunorubicin Hydrochloride.

Assay Perform the test as directed in the Assay under Daunorubicin Hydrochloride. Weigh accurately a portion of Daunorubicin Hydrochloride for Injection, equivalent to about 20 mg (potency) according to the labeled potency, add 4.0 mL of the internal standard solution, add the mobile phase to make exactly 20 mL and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Deferoxamine Mesilate



$C_{25}H_{46}N_6O_8\cdot CH_4O_3S$: 656.79

N-[5-[[4-[5-[Acetyl(hydroxy)amino]pentylamino]-4-oxobutanoyl]-hydroxyamino]pentyl]-*N*′-(5-aminopentyl)-*N*′-hydroxybutanediamide; methanesulfonic acid [*138-14-7*]

Deferoxamine Mesilate contains not less than 98.0 % and not more than 102.0 % of deferoxamine mesilate ($C_{25}H_{46}N_6O_8\cdot CH_4O_3S$), calculated on the anhydrous basis.

Description Deferoxamine Mesilate is a white to pale yellow, crystalline powder.
Deferoxamine Mesilate is freely soluble in water and practically insoluble in ethanol (99.5), in 2-propanol or in ether.

Melting point—About 147 °C (with decomposition).

Identification (1) To 5 mL of a solution of Deferoxamine Mesilate (1 in 500), add 1 drop of iron (III) chloride TS: a deep red color is observed.

(2) Deferoxamine Mesilate responds to the Qualitative Tests (1) for mesilate.
(3) Determine the infrared spectra of Deferoxamine Mesilate and Deferoxamine Mesilate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Chloride***—Perform the test with 1.0 g of Deferoxamine Mesilate. Prepare the control solution with 0.90 mL of 0.01 mol/L hydrochloric acid VS (not

more than 0.032 %).

(3) ***Sulfate***—Perform the test with 0.6 g of Deferoxamine Mesilate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Deferoxamine Mesilate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Deferoxamine Mesilate according to Method 3 and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(6) ***Related substances***—Dissolve 50 mg of Deferoxamine Mesilate in 50 mL of the mobile phase and use this solution as the test solution. Pipet 3.0 mL of the test solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: the total area of all peaks other than the peak of deferoxamine from the test solution is not larger than the peak area of deferoxamine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 1.32 g of dibasic ammonium phosphate, 0.37 g of disodium ethylenediaminetetraacetate dihydrate and 1.08 g of sodium 1-heptanesulfonate in 950 mL of water. Adjust pH of this solution with phosphoric acid to 2.8 and add 100 mL of 2-propanol to 800 mL of this solution.

Flow rate: Adjust the flow rate so that the retention time of deferoxamine is about 15 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of deferoxamine from 20 μL of the standard solution is between 5 mm and 20 mm.

System performance: Dissolve 16 mg of Deferoxamine Mesilate and 4 mg of methylparahydroxybenzoate in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, deferoxamine and methylparahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 4.0.

Time span of measurement: About twice as long as the retention time of deferoxamine after the solvent peak.

Water Not more than 2.0 % (0.2 g, volumetric titration, direct titration).

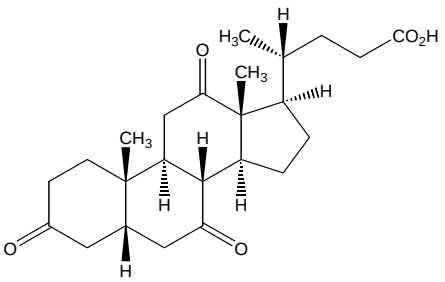
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 60 mg of Deferoxamine Mesilate and Deferoxamine Mesilate RS (determine the content of water before using in the same manner as Deferoxamine Mesilate), dissolve each in 20 mL of water, add 10 mL of 0.05 mol/L sulfuric acid TS and add water to make exactly 50 mL. Pipet 5.0 mL each of these solutions, add exactly 5 mL of 0.05 mol/L sulfuric acid TS and exactly 0.2 mL of iron (III) chloride TS, then add water to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under the Spectrophotometry, using a solution prepared by adding 0.05 mol/L sulfuric acid TS to 0.2 mL of ferric chloride (III) TS to make exactly 50 mL as the blank and determine the absorbances, A_T and A_S, for the test solution and the standard solution, respectively, at 430 nm.

$$\text{Amount (mg) of deferoxamine mesilate } (C_{25}H_{46}N_6O_8{\cdot}CH_4O_3S) = \text{Amount (mg) of Deferoxamine Mesilate RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers

Dehydrocholic Acid



$C_{24}H_{34}O_5$: 402.52

(4*R*)-4-[(5*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-Di-methyl-3,7,12-trioxo-1,2,4,5,6,8,9,11,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-17-yl] pentanoic acid [*81-23-2*]

Dehydrocholic Acid, when dried, contains not less than 98.5 % and not more than 101.0 % of dehydrocholic acid ($C_{24}H_{34}O_5$).

Description Dehydrocholic Acid is a white crystalline powder, is odorless and has a bitter taste.
Dehydrocholic Acid is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Dehydrocholic Acid dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde, allow to stand for 5 minutes and add 5 mL of water: the solution shows a yellow color and a blue-green fluorescence.
(2) To 20 mg of Dehydrocholic Acid, add 1 mL of ethanol (95), shake, add 5 drops of *m*-dinitrobezene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8) and allow to stand: a purple to red-purple color is observed and gradually changes to brown.

Specific Optical Rotation $[\alpha]_D^{20}$: +29 ~ +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting Point 233 ~ 242 °C.

Purity (1) ***Odor***—To 2.0 g of Dehydrocholic Acid, add 100 mL of water and boil for 2 minutes: the solution is odorless.

(2) ***Clarity and color of solution***—To 0.10 g of Dehydrocholic Acid, previously powdered, add 30 mL of ethanol (95) and dissolve by shaking for 10 minutes: the solution is clear and colorless.

(3) ***Chloride***—To 2.0 g of Dehydrocholic Acid, add 100 mL of water, shake for 5 minutes and filter. Take 25 mL of the filtrate, add 6 mL of dilute nitric acid, heat in a water-bath for 6 minutes, filter after cooling and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(4) ***Sulfate***—Add 1 mL of dilute hydrochloric acid to 25 mL of the test solution obtained in (3), heat in a water-bath for 6 minutes, filter after cooling and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.5 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(5) ***Heavy metals***—Proceed with 1.0 g of Dehydrocholic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Barium***—To the solution obtained in (1), add 2 mL of hydrochloric acid and boil for 2 minutes. Cool, filter and wash with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate, add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

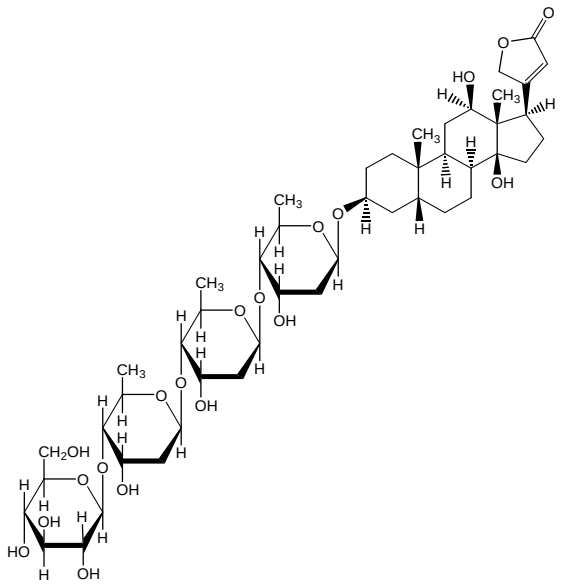
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.5 g of Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water and dissolve by warming. Add 2 drops of phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS, add 100 mL of freshly boiled and cooled water as the end point is approached and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and Storage ***Containers***—Well-closed containers.

Deslanoside



$C_{47}H_{74}O_{19}$: 943.08

3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-12,14-Dihydroxy-3-[(2*R*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-5-[(2*S*,4*S*,5*S*,6*R*)-4-hydroxy-6-methyl-5-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-6-methyloxan-2-yl]oxy-6-methyloxan-2-yl]oxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2*H*-furan-5-one [*17598-65-1*]

Deslanoside, when dried, contains not less than 90.0 % and not more than 102.0 % of deslanoside ($C_{47}H_{74}O_{19}$).

Description Deslanoside appears as colorless or white crystals or white crystalline powder.
Deslanoside is freely soluble in anhydrous pyridine,

sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Deslanoside is hygroscopic.

Identification Transfer 1 mg of Deslanoside to a small test tube, about 10 mm in internal diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10000) and underlay gently with 1 mL of sulfuric acid: at the zone of contact of two liquids, a brown ring is produced and the color of the upper layer near to the contact zone changes gradually to blue through purple and the entire acetic acid (100) layer changes to a blue-green color through a deep blue color.

Specific Optical Rotation $[\alpha]_D^{20}$: +6.5 ~ +8.5° (after drying, 0.5 g, anhydrous pyridine, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***— Dissolve 20 mg of Deslanoside in 10 mL of ethanol (95) and 3 mL of water by warming, cool and dilute to 100 mL with water: the solution is clear and colorless.

(2) ***Related substances***—Dissolve 10 mg of Deslanoside in exactly 5 mL of methanol and use this solution as the test solution. Dissolve 1.0 mg of Deslanoside RS in exactly 5 mL of methanol and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84 : 15 : 1) to a distance of about 13 cm and air-dry the plate. Spray evenly dilute sulfuric acid on the plate and heat the plate at 110 °C for 10 minutes: the spots other than the principal spot from the test solution are not larger than and more intense than the spot from the standard solution.

Loss on Drying Not more than 8.0 % (0.5 g, in vacuum, P_2O_5, 60 °C, 4 hours).

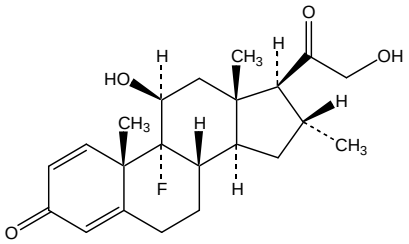
Residue on Ignition Not more than 0.5 % (0.1 g).

Assay Dissolve about 12 mg each of Deslanoside and Deslanoside RS, previously dried and accurately weighed, in 20 mL each of methanol, add water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Pipet 5.0 mL each of the test solution and the standard solution, transfer to light-resistant, volumetric flasks, shake well with 5 mL each of 2,4,6-trinitrophenol TS and 0.5 mL each of a solution of sodium hydroxide (1 in 10), diluted methanol (1 in 4) to make exactly 25 mL and allow to stand at a temperature between 18 °C and 22 °C for 25 minutes. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 485 nm as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of diluted methanol (1 in 5) in the same manner as the blank.

$$\text{Amount (mg) of deslanoside } (C_{47}H_{74}O_{19}) = \text{Amount (mg) of Deslanoside RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Desoximetasone



$C_{22}H_{29}FO_4$: 376.46

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*382-67-2*]

Desoximetasone contains not less than 97.0 % and not more than 103.0 % of desoximetasone ($C_{22}H_{29}FO_4$), calculated on the dried basis.

Description Desoximetasone is a white, crystalline powder.
Desoximetasone is very soluble in ethanol (95), in acetone or in chloroform and practically insoluble in water.

Identification (1) Determine the infrared spectra of Desoximetasone and Desoximetasone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 0.1 g of Desoximetasone in a mixture of chloroform and ethanol (95) (3:1) to make 10 mL and use this solution as the test solution. Separately, dissolve 0.1 g of Desoximetasone RS in a mixture of chloroform and ethanol (95) (3:1) to make 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Expose the plate with ultraviolet light (main wavelength 254 nm) and spray evenly the plate with *p*-toluenesulfonic acid hy-

drate in ethanol (95) (1 in 5): the R_f value of the principal spot obtained from the test solution corresponds to that from the standard solution.

Specific Optical Rotation $[\alpha]_D^{25}$: +107 ~ -112° (0.1 g, after drying, chloroform, 20 mL, 100 mm).

Melting Point 206 ~ 218 °C, but the range between beginning and end of melting does not exceed 4 °C.

Purity ***Heavy metals***—Proceed with 1.0 g of Desoximetasone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 1.0 % (1 g, 105 °C, constant mass).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 40 mg of Desoximetasone, add methanol to make exactly 100 mL, pipet 10.0 mL of this solution, add a mixture of methanol and acetonitrile (1:1) to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Desoximetasone RS, add methanol to make exactly 50 mL, pipet 10.0 mL of this solution, add a mixture of methanol and acetonitrile (1:1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the height of the desoximetasone peak in the test solution, H_T, and in the standard solution, H_S.

$$\text{Amount (mg) of desoximetasone } (C_{22}H_{29}FO_4) = \text{Amount (mg) of Desoximetasone RS} \times \frac{H_T}{H_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (3-10 μm in particle diameter).

Mobile phase: A mixture of methanol, water and acetic acid (100) (65:35:1).

Flow rate: 1 mL/minute.

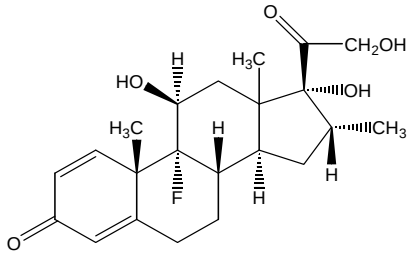
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor for desoximetasone peak is not more than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the heights of desoximetasone peak is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers

Dexamethasone



$C_{22}H_{29}FO_5$: 392.46

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*50-02-2*]

Dexamethasone, when dried, contains not less than 97.0 % and not more than 102.0 % of dexamethasone ($C_{22}H_{29}FO_5$).

Description Dexamethasone appears as white to pale yellow crystals or crystalline powder and is odorless. Dexamethasone is sparingly soluble in methanol, in ethanol (95) or in acetone and practically insoluble in water.

Melting point—About 245 °C (with decomposition).

Identification (1) Proceed with 10 mg of Dexamethasone as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests for fluoride.

(2) Dissolve separately 1.0 mg of Dexamethasone and Dexamethansone RS in 10 mL of ethanol (95), pipet 2.0 mL each of these solutions, add 10 mL each of phenylhydrazine hydrochloride TS, shake and warm on a water-bath at 60 °C for 20 minutes. Cool and determine the absorption spectra with these solutions as directed under Ultraviolet-visible Spectrophotometry using a solution, prepared with 2.0 mL of ethanol (95) in the same manner, as the blank: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Dexamethasone and Dexamethasone RS, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve Dexamethasone and

Dexamethasone RS in acetone, respectively, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +86 ~ +94° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Dexamethasone according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related Substances***—Dissolve 0.10 g of Dexamethasone in 10 mL of acetone and use this solution as the test solution. Pipet 2 mL of the test solution, add a solution, prepared by dissolving 1.32 g of ammonium formate in water to make 1000 mL and adjusted to pH 3.6 with formic acid, to make 100 mL, and use this solution as the test solution. To exactly 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the peak area other than dexamethasone is not larger than the peak area of dexamethasone obtained from the standard solution, and the total area of the peaks other than the peak of dexamethasone from the test solution is not larger than 2 times the peak area of dexamethasone from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 1.32 g of ammonium formate in 1000 mL of water, and adjust the pH to 3.6 with formic acid. To 670 mL of this solution, add 330 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 13 minutes.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of dexamethasone obtained with 10 μL of this solution is equivalent to 8 to 12 % of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dexamethasone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dexamethasone is not more than 1.0 %.

Time span of measurement: About 4 times as long as the retention time of dexamethasone beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (0.2 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.2 g, platinum crucible).

Assay Dissolve about 10 mg each of Dexamethasone and Dexamethasone RS, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of dexamethasone of the test solution and the standard solution to that of the internal standard, respectively.

$$\text{Amount (mg) of dexamethasone } (C_{22}H_{29}FO_5) = \text{Amount (mg) of Dexamethasone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and acetonitrile (2 : 1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, dexamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

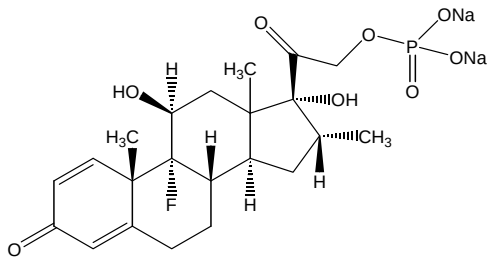
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dexame-

thasone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dexamethasone Phosphate Disodium



Dexamethasone Disodium Phosphate
Dexamethasone Sodium Phosphate

$C_{22}H_{28}FNa_2O_8P$: 516.41

Sodium 2-[(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] phosphate [*2392-39-4*]

Dexamethasone Phosphate Disodium contains not less than 97.0 % and not more than 102.0 % of dexamethasone phosphate disodium ($C_{22}H_{28}FNa_2O_8P$), calculated on the anhydrous and ethanol-free basis.

Description Dexamethasone Phosphate Disodium is a white to pale yellow crystalline powder and is odorless or has slightly ethanol-like smell.

Dexamethasone Phosphate Disodium is freely soluble in water, slightly soluble in ethanol (95), very slightly soluble in 1,4-dioxane and practically insoluble in chloroform and in ether.

Dexamethasone Phosphate Disodium is very hygroscopic.

Identification (1) Transfer 20 mg of Dexamethasone Phosphate Disodium to a centrifugal tube, add 5 mL of alkaline phosphatase TS, vigorously shake and allow to stand for 30 minutes. Add 5 mL of ethyl acetate, vigorously shake, centrifuge and use the ethyl acetate layer (upper layer) as the test solution. Separately, transfer 15 mg of Dexamethasone RS to a volumetric flask, add ethyl acetate to dissolve, add ethylacetate to make 5 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate in a mixture of chloroform, methanol and water (180 : 15 : 1) to a distance of about 15 cm and air-dry the plate. Observe under ultra-violet light (main wavelength: 254 nm). The R_f value of the principal spot obtained from the test solution corresponds to the that obtained from the standard solution.

(2) The residue from ignition of Dexamethasone Phosphate Disodium responds to the Qualitative Tests for sodium and for phosphate.

pH Between 7.5 and 10.5 in a solution (1 in 100).

Specific Optical Rotation $[\alpha]_D^{20}$: +74 ~ +82° (calculated on the anhydrous and ethanol-free basic, 0.1 g, water, 10 mL, 100 mm).

Purity (1) ***Ethanol***—Weigh about 0.5 g of Dexamethasone Phosphate Disodium, accurately weighed, to a volumetric flask, add exactly 5 mL of the internal standard solution to dissolve, then add the water to make exactly 10 mL. After shaking, use this solution as the test solution. Separately, pipet diluted ethanol (1 in 50), determine the specific gravity at 25 °C, calculate the amount of ethanol and use this solution as the standard stock solution. Pipet 4.0 mL of this solution to a volumetric flask, add exactly 5 mL of the internal standard solution and add water to make exactly 10 mL, shake and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions and determine the peak area ratios, Q_T and Q_S, of ethanol for the test solution and the standard solution, respectively, to that of the internal standard: the amount of ethanol (C_2H_5OH) in Dexamethasone Phosphate Disodium is not more than 8.0 %.

$$\text{Amount (\%) of ethanol } (C_2H_5OH) = 4 \times \frac{S}{W} \times \frac{Q_T}{Q_S}$$

S: Amount (%) of ethanol in the standard stock solution

W: Amount (g) of Dexamethasone Phosphate Disodium in the test solution

Internal standard solution—A mixture of 2-propanol and water (1 in 100)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A glass column, about 4 mm in internal diameter and about 1.8 m in length, packed with copolymer of 4-vinyl pyridine and styrenedivinyl benzene (80 - 100 mesh in particle diameter).

Column temperature: A constant temperature of about 120 °C

Carrier gas: Nitrogen

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the resolution between the peaks of

ethanol and internal standard is not less than 2.0 and symmetry factor for ethanol is not more than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak height of ethanol to that of the internal standard is not more than 4.0 %.

(2) ***Phosphate ion***—Dissolve about 50 mg of Dexamethasone Phosphate Disodium, accurately weighed, in a mixture of 10 mL of water and 5 mL of 1 mol/L sulfuric acid TS in a volumetric flask, by warming, if necessary. Add 1 mL of each of phosphate reagent A and phosphate reagent B, dilute with water to make exactly 25 mL, mix, allow to stand at room temperature for 30 minutes and use this solution as the test solution. Separately, using 5.0 mL of phosphate standard solution, proceed in the same manner as the preparation of the test solution and use this solution as the standard solution. Determine absorbances of the test solution and the standard solution at 730 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. The absorbance of the test solution is not more than that of the standard solution (not more than 1.0 % of phosphate).

Phosphate standard solution—Dissolve 143.3 mg of dried monobasic potassium phosphate, KH_2PO_4, in water to make 1000 mL (0.10 mg PO_4/mL).

Phosphate reagent A—Dissolve 5 g of hexammonium heptamolybdate tetrahydrate in 0.5 mol/L sulfuric acid TS to make 100 mL.

Phosphate reagent B—Dissolve 0.35 g of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium sulfite heptahydrate, mix to dissolve and add water to make 100 mL.

(3) ***Free Dexamethasone***—Separately inject 20 μL of the standard solution and the test solution under the Assay, respectively. Determine the peak areas, A_T and A_S of Dexamethasone of the test solution and the standard solution, respectively. (not more than 1.0 %).

$$\text{Amount (μg) of dexamethasone } (C_{22}H_{29}FO_5) = 1000 \times C \times \frac{A_T}{A_S}$$

C: Concentration of the Dexamethasone RS in the standard solution (μg/mL).

(4) ***Related substances***—Weigh accurately 25 mg of Dexamethasone Phosphate Disodium, dissolve in mobile phase A to make 25 mL and use this solution as the test solution. Perform the test with 15 μL of the test solution as directed in the area percentage method under Liquid Chromatography according to the following operating conditions: the content of each related substance is not more than 1.0 % and the content of all related substances is not more than 2.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i : Peak area of each related substance from the test solution

A_S : Sum of all peak areas from the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water, methanol and buffer solution (7:7:6)

Mobile phase B: A mixture of methanol and buffer solution (7:3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	90	10
0-3.5	90	10
3.5-23.5	90→60	10→40
23.5-34.5	60→5	40→95
34.5-59.5	5	95
59.5-60	5→90	95→10

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 15 μL of the test solution under the above operating conditions, the resolution between the main peak and the nearest related substance peak is not less than 1.0.

System repeatability: When the test is repeated 6 times with 15 μL each of the test solution, the relative standard deviation of the peak area is not more than 4.0.

Buffer solution—Dissolve 7.0 g of ammonium acetate in 1000 mL of water and adjust the pH to 4.0 with acetic acid (100).

Water Not more than 16.0 % (including ethanol content, 0.4 g, volumetric titration, direct titration).

Assay Take accurately about 50 mg of Dexamethasone Phosphate Disodium, dissolve in mobile phase to make exactly 100 mL. Pipet 5.0 mL of this solution and add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, dissolve a portion of Dexamethasone Phospahte RS, pre-

viously dried at 40 °C and 0.67 kPa, in mobile phase to make the concentration of 0.5 mg of Dexamethasone Phospahte per mL and use this solution as the standard solution (1). Weigh accurately a portion of Dexamethasone RS, previously dried at 105 °C for 3 hours, dissolve in a mixture of water and methanol (1 : 1) to make the concentration of 50 μg of Dexamethasone RS per mL and use this solution as the standard solution (2). Pipet 10.0 mL of the standard solution (1) and 1.0 mL of the standard solution (2), add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of dexamethasone phosphate of the test solution and the standard solution, respectively.

Amount (mg) of dexamethasone phosphate disodium ($C_{22}H_{28}FNa_2O_8P$) $= \dfrac{516.41}{472.45} \times C \times \dfrac{A_T}{A_S}$

C: Concentration of the Dexamethasone Phospahte RS in the standard solution (μg/mL).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.5 mm in internal diameter and about 25 cm in length, packed with phenylatedsilica gel for liquid chromatography.

Mobile phase: To 7.5 mL of the triethylamine, add water to make 1000 mL, and adjust the pH to 5.4 with phosphoric acid. Mix this solution and methanol at the ratio of 74:26.

Flow rate: 1.2 mL/minute.

System suitability

System performance : When the procedure is run with 10 μL of the standard solution under the above operating conditions, and calculate the resolution, dexamethasone and dexamethasone phosphate are eluted in this order and the resolution between the dexamethasone peak and dexamethasone phosphate peak is not less than 1.8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Dexamethasone Phosphate Disodium Injection

Dexamethasone Disodium Phosphate Injection

Dexamethasone Phosphate Disodium Injection is an aqueous solution for Injection.

Dexamethasone Phosphate Disodium Injection contains not less than 90.0 % and not more than 115.0 % of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$: 472.45).

Method of Preparation Prepare as directed under Injections with the Dexamethasone Phosphate Disodium.

Description Dexamethasone Phosphate Disodium Injection is a clear, colorless liquid.

Identification Pipet a volume of Dexamethasone Phosphate Disodium Injection, equivalent to about 10 mg of dexamethasone phosphate, into a volumetric flask, add water to make 100 mL and mix. Pipet 5 mL of this solution into a separatory funnel and wash with two 10 mL volumes of water-washed methylene chloride, discarding the washings. Transfer the solution into a glass-stoppered tube and add 5 mL of alkaline phosphatase solution, prepared by dissolving 50 mg of alkaline phosphatase in 50 mL of pH 9 buffer with magnesium. Allow to stand at 37 °C for 45 minutes and extract with 25 mL of methylene chloride. Evaporate 15 mL of the methylene chloride extract in a steam-bath to dryness and dissolve the residue in 1 mL of methylene chloride and use this solution as the test solution. Separately, dissolve a portion of Dexamethasone RS in methylene chloride to contain 300 μg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography, respectively. Develop the plate with a mixture of acetone, chloroform, water (50 : 50 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly diluted sulfuric acid (1in 2) upon the plate and heat the plate at 105 °C until black or brown spots appear. The spot from the test solution shows the same R_f value as the spot from the standard solution.

pH 7.0 ~ 8.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 31.3 EU/mg of dexamethasone phosphate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement

Determination of Volume of Injection in Containers It meets the requirement.

Assay Transfer an accurately measured volume of Dexmethasone Phosphate Disodium Injection, equivalent to about 8 mg of dexamethasone phosphate ($C_{22}H_{30}FO_8P$), to a volumetric flask. Dilute with mobile phase to make 100 mL and mix and use this solution as test solution. Separately, weigh accurately a portion of dexamethasone phosphate RS, previously dried at 40 °C under 0.67 kPa, dissolve in mobile phase to make the concentration of 80 μg of dexamethasone phosphate per mL and use this solution as the standard solution. Prepare this solution at the time of use. Perform the test with 20 μl each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area, A_T and A_S, of the dexamethasone of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of dexamethasone phosphate } (C_{22}H_{30}FO_8P) = \frac{0.1 \times C}{V} \times \frac{A_T}{A_S}$$

C: Concentration of the dexamethasone phosphate RS in the standard solution (μg/mL),
V: Volume (mL) of sample taken

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A 0.01 mol/L monobasic potassium phosphate solution using a mixture of water and methanol (1 : 1) as the solvent.

Flow rate: 1.6 mL/minute.

System suitability

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dexamethasone phosphate is not more than 1.5 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Dexamethasone Tablets

Dexamethasone Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$: 392.47).

Method of Preparation Prepare as directed under Tablets, with Dexamethasone.

Identification Evaporate 10 mL of the methanol extract of Dexamethsone Tablets, obtained as directed under preparation of the test solution in the Assay on a water-bath to dryness, dissolve the residue in 1 mL of chloroform and use this solution as the test solution. Separately, weigh a quantity of Dexamethasone RS and dissolve in chloroform to contain 500 μg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL of the test solution and 20 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methylene chloride and methanol (45 : 4) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254nm): the R_f values of the principal spots obtained from the test solution and the standard solution are the same.

Dissolution Test Perform the test with 1 tablet of Dexamethasone Tablets at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 500 mL of diluted hydrochloric acid (1 in 100). After 45 minutes of the start of the test, extract a filtered aliquot of dissolution solution, equivalent to about 200 μg of Dexamethasone, with three 15 mL volumes of chloroform. Evaporate the combined chloroform extract in a water-bath to dryness, cool, dissolve the residue in 20 mL of ethanol (95) and use this solution as the test solution. Separately weigh a quantity of Dexamethasone RS, previously dried at 105 °C for 3 hours, dissolve in methanol to contain 10 μg per mL and use this solution as the standard solution. Transfer 20 mL each of the test solution and the standard solution to an Erlenmeyer flask, add 2.0 mL of a solution, dissolve 50 mg of blue tetrazolium in 10 mL of methanol. Use 20 mL ethanol (95) for blank test. Add 2 mL of a mixture of ethanol (95) and tetramethylammonium hydroxide TS (9:1) to each of these flasks and mix. Immediately determine the absorbance, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorption around 525 nm as directed under Ultraviolet-visible Spectrophotometry in contrast with blank solution.

$$\text{Amount (mg) of dexamethasone } (C_{22}H_{29}FO_5) = 10 \times C \times \frac{1}{V} \times \frac{A_T}{A_S}$$

C: Concentration of standard solution (μg/mL),
V: Volume of the aliquot extracted with chloroform (mL).

Not less than 70 % of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) is dissolved in 45 minutes.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure. Place 1 tablet of Dexamethasone Tablets in a separatory funnel with 15

mL of water and swirl to dissolve the tablet completely. Extract with four 10 mL volumes of chloroform, filter each volume through chloroform-washed cotton into a volumetric flask, add chloroform to make 50 mL and mix. Pipet a volume of this solution, equivalent to about 200 μg of Dexamethasone into a glass-stoppered Erlenmeyer flask, evaporate the chloroform on a water-bath to dryness, cool and dissolve the residue in 20.0 mL of ethanol (95). Separately pipet 20 mL of standard solution in Dissolution test into a stoppered Erlenmeyer flask. Calculate the amount of Dexamethasone in the same manner of Dissolution Test.

$$\text{Amount (mg) of dexamethasone } (C_{22}H_{29}FO_5) = \frac{C}{V} \times \frac{A_T}{A_S}$$

C: Concentration of standard solution (μg/mL),
V: Volume of the solution, equivalent to 200 μg of Dexamethasone (mL).

Assay Weigh and finely powder not less than 20 Dexamethasone Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of Dexamethasone, transfer to a 100 mL volumetric flask and add 30 mL of diluted methanol (1 in 2). After shaking for 30 minutes, add 5 mL exactly of the internal standard solution, diluted methanol (1 in 2) to make 100 mL and mix. Filter and use the filtrate as the test solution. Separately, weigh accurately about 10 mg of Dexamethasone RS, transfer to a 100 mL volumetric flask and add 30 mL of diluted methanol (1 in 2). Add 5 mL of the internal standard solution, diluted methanol (1 in 2) to make 100 mL, mix and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the peak area ratios, Q_T and Q_S, of dexamethasone for the test solution and the standard solution to the internal standard, respectively.

$$\text{Amount (mg) of dexamethasone } (C_{22}H_{29}FO_5) = \text{Amount (mg) of Dexamethasone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl *p*-oxybenzoate in a mixture of water and methanol (1:1) (1 in 1000)

Operating conditions

Use the operating conditions as directed under the Assay of Dexamethasone.

System suitability

System performance : Dissolve 2 mg of methyl *p*-oxybenzoate and 4 mg of propyl *p*-oxybenzoate in 100 mL of a mixture of water and methanol (1:1). When the test is performed with 10 mL of the solution as directed under the above operating conditions, methyl *p*-oxybenzoate and propyl *p*-oxybenzoate are eluted in this order with the resolution between their peaks being not less than 5.0.

System repeatability: When the test is repeated 6 times with 10 mL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dexamethason to the peak area of the internal standard is not more than 3.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Dextran 40

[*9004-54-0*]

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*) and the average molecular weight is about 40000.
Dextran 40, when dried, contains not less than 98.0 % and not more than 102.0 % of dextran.

Description Dextran 40 is a white, amorphous powder, odorless and tasteless**.**
Dextran 40 is practically insoluble in ethanol (95) or in ether.
Dextran 40 dissolves gradually in water.
Dextran 40 is hygroscopic.

Identification Take 1 mL of a solution of Dextran 40 (1 in 3000) and add 2 mL of anthrone TS: a blue-green color is observed and turns gradually dark blue-green. Then to this solution, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

Specific Optical Rotation $[\alpha]_D^{20}$: +195 ~ +203° (0.2 g, water, 10 mL, 100 mm, dissolve by warming in a water-bath if necessary).

pH Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 2.0 g of Dextran 40. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Dextran 40 according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.5 g of Dextran 40 according to Method 1 and perform the test

(not more than 1.3 ppm).

(5) ***Nitrogen***—Weigh accurately about 2 g of Dextran 40, previously dried, and perform the test as directed under the Nitrogen Determination, where 10 mL of sulfuric acid is used for decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.007) is not more than 0.010 %.

(6) ***Reducing substances***—Weigh exactly 3.00 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL and use this solution as the test solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 500 mL and use this solution as the standard solution. Pipet 5.0 mL each of the test solution and the standard solutions and add water to make exactly 50 mL. Pipet 5.0 mL each of these solutions, add 5.0 mL of alkaline copper TS and heat for 15 minutes in a water-bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). The titrant consumed for the test solution is not less than that for the standard solution.

Loss on Drying Not more than 5.0 % (1 g, 105 °C, 6 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Bacterial Endotoxins Less than 2.5 EU/g, when Dextran 40 is used in a sterile preparation.

Antigenicity Dissolve 10.0 g of Dextran 40 in Isotonic Sodium Chloride Injection to make 100 mL, sterilize and use this solution as the test solution. Inject 1.0 mL of the test solution on three occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 g to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the test solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and each of the remaining 2 guinea pigs 21 days after the injection and inject 0.20 mL of horse serum intravenously in the same manner each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above. All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g and the total combined yeasts/mould count is not more than 100 CFU/g, when used in an injection preparation. *Escherichia coli*, Salmonella, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

Viscosity (1) ***Dextran 40***—Weigh accurately about 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL and use this solution as the test solution. Perform the test with the test solution and with water as directed in Method 1 under the Viscosity Determination at 25 °C: the intrinsic viscosity is between 0.16 and 0.19.

(2) ***High-molecular fraction***—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL and transfer to a flask. Add slowly enough methanol to obtain 7 % to 10 % of the precipitate (usually 80 to 90 mL) at 25 ± 1 °C with stirring. Dissolve the precipitate at 35 °C in a water-bath with occasional shaking and allow to stand for more than 15 hours at 25 ± 1 °C. Remove the clear supernatant liquid by decantation and heat the precipitate of the lower layer to dryness on a water-bath. Dry the residue and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) ***Low-molecular fraction***—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL and transfer to a flask. Add slowly enough methanol to obtain 90 % to 93 % of the precipitate (usually 115 to 135 mL) at 25 ± 1 °C with stirring, centrifuge at 25 °C and evaporate the clear supernatant liquid to dryness on a water-bath. Dry the residue and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL and use this solution as the test solution. Determine the optical rotation with the test solution as directed under the Optical Rotation Determination in a 100 mm cell at 20 ± 1 °C.

$$\text{Amount (mg) of Dextran 40} = \alpha_D \times 253.8$$

Containers and Storage ***Containers***—Tight containers.

Dextran 40 Injection

Dextran 40 Injection is an aqueous solution for injection. Dextran 40 Injection contains not less than 9.5 w/v % and not more than 10.5 w/v % of Dextran 40.

Method of Preparation

Dextran 40	10 g
Isotonic Sodium Chloride Injection	a sufficient quantity
To make	100 mL

Prepare as directed under Injections, with the above

ingredients. No preservative is added.

Description Dextran 40 Injection is a clear and colorless liquid, and is slightly viscous.

Identification (1) Dilute 1 mL of Dextran 40 Injection with water to make 200 mL, pipet 1 mL of this solution and add 2 mL of anthrone TS: a blue-green color is observed and turns gradually dark blue-green. Then to this solution, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative Tests for sodium salt and for chloride.

pH 4.5 ~ 7.0.

Purity (1) ***5-Hydroxymethylfurfurals***—Take a volume of Dextran 40 Injection, equivalent to 1.0 g of Dextran 40 according to the labeled amount, add water to make exactly 500 mL. Determine the absorbance of this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 284 nm is not more than 0.25.

(2) ***Heavy metals***—Proceed with 0.2 g of Dextran 40 Injection according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.5 EU/mL of Dextran 40 Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Viscosity Measure exactly 2 mL to 5 mL of Dextran 40 Injection, add Isotonic Sodium Chloride Injection to make exactly 100 mL and use this solution as the test solution. Perform the test with the test solution and with Isotonic Sodium Chloride Injection as directed in Method 1 under the Viscosity Determination at 25 °C: the intrinsic viscosity is between 0.16 and 0.19. Calculate the concentration of the test solution (g/100 mL) as directed in the Assay.

Assay Take exactly 30 mL of Dextran 40 Injection, add water to make exactly 50 mL and use this solution as the test solution. Determine the optical rotation, α_D, with the test solution as directed under the Optical Rotation Determination in a 100-mm cell at 20 ± 1 °C.

$$\text{Amount (mg) of Dextran 40 in 100 mL of Dextran 40 Injection} = \alpha_D \times 846.0$$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Srotage—Avoid exposure to undue fluctuations in temperature.

Dextran 70

[*9004-54-0*]

Dextran 70 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*) and the average molecular weight is about 70000.
Dextran 70, when dried, contains not less than 98.0 % and not more than 102.0 % of Dextran 70.

Description Dextran 70 is a white, amorphous powder and is odorless and tasteless.
Dextran 70 is practically insoluble in ethanol (95) or in ether.
Dextran 70 dissolves gradually in water.
Dextran 70 is hygroscopic.

Identification Perform the test according to Identification of Dextran 40.

Specific Optical Rotation $[\alpha]_D^{20}$: +195 ~ +203° (0.2 g, water, 10 mL, 100 mm, dissolve by warming in a water-bath if necessary).

pH Dissolve 3.0 g of Dextran 70 in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) **Clarity and color of solution**—Dissolve 1.0 g of Dextran 70 in 10 mL of water with warming: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 2.0 g of Dextran 70. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid (not more than 0.018 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Dextran 70 according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.5 g of Dextran 70 according to Method 1 and perform the test (not more than 1.3 ppm).

(5) ***Nitrogen***—Weigh accurately about 2 g of Dextran 70, previously dried, perform the test as directed under the Nitrogen Determination, where 10 mL of sulfuric acid is used for decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of Nitrogen (N: 14.007) is not more than 0.010 %.

(6) ***Reducing substances***—Weigh exactly 3.00 g of

Dextran 70, previously dried, dissolve in water to make exactly 50 mL and use this solution as the test solution. Separately, weigh exactly 0.300 g of glucose, previously dried, dissolve in water to make exactly 500 mL and use this solution as the standard solution. Pipet 5.0 mL each of the test solution and the standard solution and add water to make exactly 50 mL, respectively. Pipet 5.0 mL each of these solutions, add 5.0 mL of alkaline copper TS and heat for 15 minutes in a water-bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). The titrant consumed for the test solution is not less than that for the standard solution.

Loss on Drying Not more than 5.0 % (1 g, 105 °C, 6 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Bacterial Endotoxins Less than 0.5 EU/mL of a solution prepared by diluting Dextran 70 to 6 w/v % with Isotonic Sodium Chloride Injection, when used in a sterile preparation.

Antigenicity Dissolve 6.0 g of Dextran 70 in Isotonic Sodium Chloride Injection to make 100 mL, sterilize and use this solution as the test solution. Perform the test according to Antigenicity under Dextran 40

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g and the total combined yeasts/mould count is not more than 100 CFU/g, when used in an injection preparation. *Escherichia coli*, Salmonella, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

Viscosity (1) ***Dextran 70***—Weigh accurately about 0.2 to 0.5 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL and use this solution as the test solution. Perform the test with the test solution and with water as directed in Method 1 under the Viscosity Determination at 25 °C: the intrinsic viscosity is between 0.21 and 0.26.

(2) ***High-molecular fraction***—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL and transfer to a flask. Add slowly enough methanol to obtain 7 % to 10 % of the precipitate (usually 75 to 85 mL) at 25 ± 1 °C with stirring. Dissolve the precipitate at 35 °C in a water-bath with occasional shaking and allow to stand for more than 15 hours at 25 ± 1 °C. Remove the clear supernatant liquid by decantation and heat the precipitate of the lower layer on a water-bath to dryness. Dry the residue and determine the intrinsic viscosity of the dried residues as directed in (1): the value is not more than 0.35.

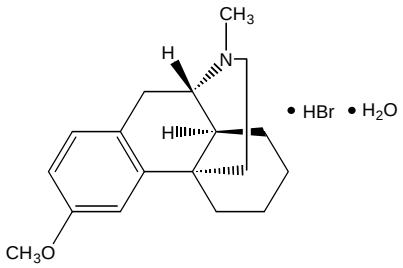
(3) ***Low-molecular fraction***—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL and transfer to a flask. Add slowly enough methanol to obtain 90 % to 93 % of the precipitate (usually 110 to 130 mL) at 25 ± 1 °C with stirring, centrifuge at 25 °C and evaporate the clear supernatant liquid to dryness on a water-bath. Dry the residue and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.10.

Assay Weigh accurately about 3 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL and use this solution as the test solution. Determine the optical rotation, α_D, as directed under the Optical Rotation Determination in a 100-mm cell at 20 ± 1 °C.

$$\text{Amount (mg) of Dextran 70} = \alpha_D \times 253.8$$

Containers and Storage ***Containers***—Tight containers.

Dextromethorphan Hydrobromide Hydrate



$C_{18}H_{25}NO \cdot HBr \cdot H_2O$: 370.32

[*6700-34-1*]

Dextromethorphan Hydrobromide Hydrate contains not less than 98.0 % and not more than 101.0 % of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$), calculated on the anhydrous basis.

Description Dextromethorphan Hydrobromide Hydrate appears as white crystals or crystalline powder.
Dextromethorphan Hydrobromide Hydrate is very soluble in methanol, freely soluble in ethanol (95) or in acetic acid (100), sparingly soluble in water and practically insoluble in ether.

Melting point—About 126 °C (insert the capillary tube into the bath preheated to 116 °C and continue the heating so that the temperature rises at a rate of about 3 °C per minute).

Identification (1) Determine the absorption spectra of solutions of Dextromethorphan Hydrobromide Hydrate and Dextromethorphan Hydrobromide Hydrate RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dextromethorphan Hydrobromide Hydrate and Dextromethorphan Hydrobromide Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Take 50 mL of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 100), add 2 drops of phenolphthalein TS and sodium hydroxide TS until a red color is absorbed. Add 50 mL of chloroform, shake and add 5 mL of dilute nitric acid to 40 mL of the water layer. This solution responds to the Qualitative Tests for bromide.

Specific Optical Rotation $[\alpha]_D^{20}$: + 26 ~ + 30° (0.34 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH Dissolve 1.0 g of Dextromethorphan Hydrobromide Hydrate in 100 mL of water: the pH of this solution is between 5.2 and 6.5.

Purity (1) ***Clarity and color of solution***— Dissolve 0.20 g of Dextromethorphan Hydrobromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) ***Dimethylaniline***—Take 0.50 g of Dextromethorphan Hydrobromide Hydrate, add 20 mL of water and dissolve by heating in a water-bath. After cooling, add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL: the solution has no more color than the following control solution.

Control solution—Dissolve 0.10 g of dimethylaniline in 400 mL of water by warming in a water-bath, cool and add water to make 500 mL. Pipet 5.0 mL of this solution and add water to make 200 mL. To 1.0 mL of this solution, add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL.

(3) ***Heavy metals***—Proceed with 1.0 g of Dextromethorphan Hydrobromide Hydrate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Phenolic compounds***—Dissolve 5 mg of Dextromethorphan Hydrobromide Hydrate in 1 drop of dilute hydrochloric acid and 1 mL of water, add 2 drops of iron (III) chloride TS and 2 drops of potassium hexacyanoferrate (III) TS, shake and allow to stand for 15 minutes: no blue-green color is observed.

(5) ***Related substances***—Dissolve 0.25 g of Dextromethorphan Hydrobromide Hydrate in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, ethyl acetate, methanol, dichloromethane and 13.5 mol/L ammonia water (55 : 20 : 13 : 10 : 2) to a distance of about 15 cm and air-dry the plate. Spray evenly bismuth potassium iodide TS on the plate and then spray evenly hydrogen peroxide TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water 4.0 ~ 5.5 % (0.2 g, volumetric titration, reverse titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Dextromethorphan Hydrobromide Hydrate, dissolve in 10 mL of acetic acid (100) and add 40 mL of acetic anhydride. Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.231 mg of $C_{18}H_{25}NO \cdot HBr$

Containers and Storage ***Containers***—Well-closed containers.

Diagnostic Sodium Citrate Solution

Diagnostic Sodium Citrate Solution contains not less than 3.3 w/v % and not more than 4.3 w/v % of sodium citrate hydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10). The requirement as described for aqueous injections under Injections are applicable.

Method of Preparation

Sodium Citrate Hydrate	38 g
Water for injection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.
No preservative is added.

Description Diagnostic Sodium Citrate Solution is a clear, colorless liquid.

Identification Diagnostic Sodium Citrate Solution responds to the Qualitative Tests for sodium salt and for citrate.

pH 7.0 ~ 8.5.

Assay Pipet 5.0 mL of Diagnostic Sodium Citrate Solution and evaporate on a water-bath to dryness. Dry the residue at 180 °C for 2 hours and dissolve in 30 mL of acetic acid (100) by warming. After cooling, titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.803 mg of $C_6H_5Na_3O_7.2H_2O$

Containers and Storage ***Containers***—Hermetic containers.

Diastase

Diastase is an enzyme mainly prepared from malt. Diastase has an amylolytic activity. Diastase contains not less than 440 starch saccharifying activity units per g. Diastase is usually diluted with suitable diluents.

Description Diastase is a pale yellow to pale brown powder**.**
Diastase is hygroscopic.

Purity ***Rancidity***—Diastase has no unpleasant or rancid odor and has no unpleasant or rancid taste.

Loss on Drying Not more than 4.0 % (1 g, 105 °C, 5 hours).

Assay (i) Substrate solution—Use potato starch TS for starch digestive activity.

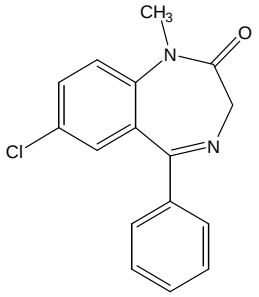
(ii) Test solution—Weigh accurately about 0.1 g of Diastase, and dissolve in water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in (i) Measurement of starch saccharifying activity of (1) Assay for starch digestive activity under the Digestion Test.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Diazepam



$C_{16}H_{13}ClN_2O$: 284.74

7-Chloro-1-methyl-5-phenyl-3*H*-1,4-benzo-diazepin-2-one [*439-14-5*]

Diazepam, when dried, contains not less than 98.0 % and not more than 101.0 % of diazepam ($C_{16}H_{13}ClN_2O$).

Description Diazepam is a white to pale yellow, crystalline powder, is odorless and has a slightly bitter taste.
Diazepam is freely soluble in acetone, soluble in ethanol (95) or in acetic anhydride, sparingly soluble in ether, slightly soluble in ethanol (99.5) and practically insoluble in water.

Identification (1) Dissolve 10 mg of Diazepam in 3 mL of sulfuric acid and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(2) Dissolve 2 mg each of Diazepam and Diazepam RS in 200 mL each of solutions of sulfuric acid in ethanol (99.5) (3 in 1000) and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Diazepam and Diazepam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Diazepam as directed under the Flame Coloration Test (2): a blue to blue-green color is observed.

Melting Point 130 ~ 134 °C.

Absorbance $E_{1\,cm}^{1\%}$ (285 nm): 425 ~ 445 [after drying , 2 mg , a solution of sulfuric acid in dehydrated ethanol (3 in 1000), 200 mL].

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Diazepam in 20 mL of ethanol (95): the solution is clear and colorless.

(2) ***Chloride***—Take 1.0 g of Diazepam, add 50 mL of water, allow to stand for 1 hour, with occasional shaking and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Diazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 1.0 g of Diazepam in 10 mL of acetone and use this solution as the test solution. Pipet 1.0 mL of the test solution and add

acetone to make exactly 100 mL. Pipet 1.0 mL of this solution, add acetone to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethylacetate and hexane (1 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.6 g of Diazepam, previously dried, dissolve in 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.474 mg of $C_{16}H_{13}ClN_2O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Diazepam Injection

Diazepam Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of diazepam ($C_{16}H_{13}ClN_2O$: 284.74).

Method of Preparation Prepare as directed under Injections, with Diazepam.

Description Diazepam Injection is a colorless, clear liquid.

Identification (1) Retention time of the principal peak in the chromatogram of the test solution under the Assay corresponds to that of the standard solution.

(2) Take Diazepam Injection, equivalent to 10 mg of Diazepam according to the labeled amount, transfer to a separatory funnel, add 20 mL of water. After shaking, add 20 mL of chloroform and shake extensively. Transfer the chloroform layer to a beaker through a filter with 5 g of anhydrous sodium sulfate. Wash anhydrous sodium sulfate with 20 mL of chloroform and add this washing to the filtrate. Evaporate the filtrate on a water-bath under the flow of air until the volume is reduced to 5 mL, take out the beaker from the water-bath and evaporate under the flow of air. Scratch and gather an oily membrane extensively by spatula and dry it in a desiccator with P_2O_5 in vacuum at 60 °C for 4 hours. Determine the infrared spectra of the residue and Diazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the similiar intensities of absorption at the same wavenumbers.

pH 6.2 ~ 6.9

Sterility Test It meets the requirement

Bacterial Endotoxins Less than 11.6 EU/mg of Diazepam.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Take accurately a volume of Diazepam Injection, equivalent to about 10 mg of Diazepam, add 10.0 mL of the internal standard solution and methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Diazepam RS add 10.0 mL of the internal standard solution and methanol to make exactly 50 mL and use this solution as the as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Calculate the ratios, Q_T and Q_S, of peak areas of Diazepam to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of diazepam } (C_{16}H_{13}ClN_2O) = \text{Amount (mg) of Diazepam RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—*p*-tolualdehyde in methanol (3 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of methanol and water (65 : 35).

Flow rate: 1.4 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution, as directed under the above operating conditions, the internal standard

and the diazepam are eluted in this order with the resolution between their peaks being not less than 5.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the ratios of peak area of diazepam to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Diazepam Tablets

Diazepam Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of diazepam ($C_{16}H_{13}ClN_2O$: 284.74).

Method of Preparation Prepare as directed under Tablets, with Diazepam.

Identification Weigh a portion of powdered Diazepam Tablets, equivalent to 50 mg of diazepam according to the labeled amount, add 50 mL of acetone, shake and filter. Take 1 mL of the filtrate, evaporate to dryness on a water-bath and dissolve the residue in 100 mL of a solution of sulfuric acid in ethanol (95) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet Spectrophotometry: it exhibits maxima between 240 nm and 244 nm, between 283 nm and 287 nm, and between 360 nm and 370 nm.

Dissolution Test Take 1 tablet of Diazepam Tablets and perform the test using 900 mL of 0.1 mol/L hydrochloric acid as dissolution solution, at 100 revolutions per minute as directed in the Method 1 under the Dissolution Test. After 30 minutes from the start of the test, take the dissolved solution, suitably dilute with dissolution solution, if necessary and use this solution as the test solution. Separately, dissolve a suitable portion of Diazepam RS to make a solution with the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at a maximum absorption wavelength of about 242 nm, as directed under Ultraviolet-visible Spectrophotometry, using 0.1 mol/L hydrochloric acid TS as the blank.
The dissolution rate of Diazepam Tablets in 30 minutes is not less than 85 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.
Take 1 tablet of Diazepam Tablets, add 5 mL of water and disintegrate by shaking. Add 30 mL of methanol, shake for 10 minutes, add methanol to make exacty 50 mL and centrifuge. Pipet V mL of the clear supernatant liquid, equivalent to 0.4 mg of diazepam ($C_{16}H_{13}ClN_2O$), add exactly 5 mL of the internal standard solution, add methanol to make 20 mL and use this solution as the test solution. Separately, weigh accurately 20 mg of Diazepam RS, previously dried at 105 °C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 20 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography under the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of diazepam to that of the internal standard.

$$\text{Amount (mg) of diazepam } (C_{16}H_{13}ClN_2O) = \text{Amount (mg) of Diazepam RS} \times \frac{Q_T}{Q_S} \times \frac{1}{V}$$

Internal standard solution—A solution of ethyl paraoxybenzoate in methanol (1 in 25000)

Operating conditions
Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak area of diazepam with respect to that of the internal standard is not more than 1.0 %.

Assay Weigh accurately not less than 20 Diazepam Tablets and make fine powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of diazepam ($C_{16}H_{13}ClN_2O$), add 10 mL of water, dissolve by shaking, add 60 mL of methanol, shake for 10 minutes, add methanol to make exactly 100 mL and centrifuge. Pipet 5 mL of the clear supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL and use this solution as the test solution. Separately, weigh accurately 50 mg of Diazepam RS, previously dried at 105 °C for 2 hours, add 10 mL of water and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of diazepam to that of the internal standard in each solution.

$$\text{Amount (mg) of diazepam } (C_{16}H_{13}ClN_2O)$$

$$= \text{Amount (mg) of Diazepam RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of ethyl paraoxybenzoate in methanol (1 in 5000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust the flow rate so that the retention time of diazepam is about 10 minutes.

System suitability

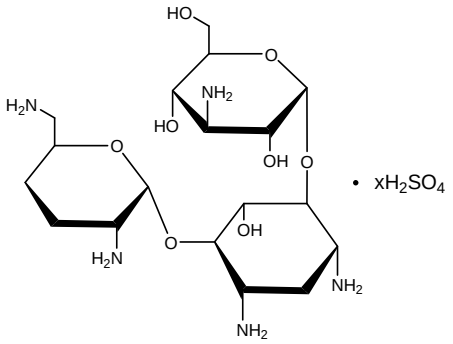
System performance: When the procedure is run with 10 μL of the standard solution, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of diazepam is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dibekacin Sulfate



$C_{18}H_{37}N_5O_8 \cdot xH_2SO_4$

(2*S*,3*R*,4*S*,5*S*,6*R*)-4-Amino-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-(aminomethyl)oxan-2-yl]oxy-2-hydroxycyclo-hexyl]oxy-6-(hydroxymethyl)oxane-3,5-diol sulfate [*58580-55-5*]

Dibekacin Sulfate is the sulfate of a derivative of bekanamycin.

Dibekacin Sulfate contains not less than 640 μg (potency) and not more than 740 μg (potency) of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52) per mg, calculated on the dried basis.

Description Dibekacin Sulfate appears as white to pale yellow powder.

Dibekacin Sulfate is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 20 mg each of Dibekacin Sulfate and Dibekacin Sulfate RS in 1 mL of water and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28) and methanol (1:1) to a distance of about 10 cm and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS and heat at 100 °C for 10 minute: the principal spots obtained from the test solution and the standard solution show a purple-brown color and the same R_f value.

(2) To 5 mL of a solution of Dibekacin Sulfate (1 in 50) add 1 drop of barium chloride TS: a white precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +96 ~ +106° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Dibekacin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Dibekacin Sulfate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 5.0 % (1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Dibekacin Suflate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of dibekacin, when Dibekacin Sulfate is used in a sterile preparation.

Assay ***The Cylinder-plate method*** (1) Culture medium Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics, having pH 6.5 to 6.6 after sterilization.

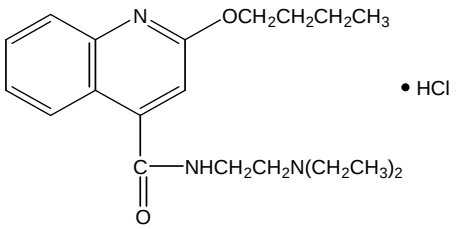
(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately an amount of Dibekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Pipet a suitable amount of this solution and dilute with 0.01 mol/L

phosphate buffer (pH 8.0) to make solutions containing 20 μg (potency) and 5 μg (potency) per mL, and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately an amount of Dibekacin Suflate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer (pH 6.0) (1 in 2) to make exactly 50 mL and use this solution as the standard stock solution. Keep the standard stock solution at 5 °C to 15 °C and use within 30 days. Pipet a suitable amount of the standard stock solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make solutions containing 20 μg (potency) and 5 μg (potency) per mL, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in 1 8) under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Dibucaine Hydrochloride



$C_{20}H_{29}N_3O_2 \cdot HCl$: 379.92

2-Butoxy-*N*-(2-(diethylamino)ethyl)quinoline-4-carboxamide hydrochloride [*61-12-1*]

Dibucaine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of dibucaine hydrochloride ($C_{20}H_{29}N_3O_2 \cdot HCl$).

Description Dibucaine Hydrochloride appears as white crystals or crystalline powder.
Dibucaine Hydrochloride is very soluble in water, in ethanol (95) or in acetic acid (100), freely soluble in acetic anhydride and practically insoluble in ether.
Dibucaine Hydrochloride is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Dibucaine Hydrochloride and Dibucaine Hydrochloride RS in 1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dibucaine Hydrochloride and Dibucaine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Dibucaine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting Point 95 ~ 100 °C. Charge Dibucaine Hydrochloride into a capillary tube for melting point determination and dry in vacuum over P_2O_5 at 80 °C for 5 hours. Seal immediately the open end of the tube and determine the melting point.

pH Dissolve 1.0 g of Dibucaine Hydrochloride in 50 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Dibucaine Hydrochloride in 20 mL of water: the solution is clear and colorless. Determine the absorbance of this solution at 430 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: it is not more than 0.03.

(2) ***Sulfate***—Perform the test with 0.30 g of Dibucaine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.056 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Dibucaine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Dibucaine Hydrochloride in 5 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of the test solution and add ethanol (95) to make exactly 20 mL. Pipet 2.0 mL of this solution, add ethanol (95) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, P_2O_5, 80 °C, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

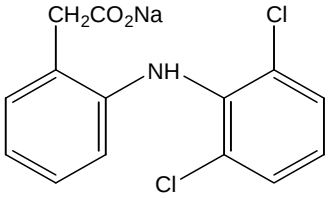
Assay Weigh accurately about 0.3 g of Dibucaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 18.996 mg of $C_{20}H_{29}N_3O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Diclofenac Sodium



$C_{14}H_{10}Cl_2NNaO_2$: 318.13

Sodium 2-(2-(2,6-dichlorophenylamino)phenyl)acetate [*15307-79-6*]

Diclofenac Sodium, when dried, contains not less than 98.5 % and not more than 101.0 % of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

Description Diclofenac sodium appears as white to pale yellowish white crystals or crystalline powder.
Diclofenac sodium is freely soluble in methanol or in ethanol (95), sparingly soluble in water or in acetic acid (100) and practically insoluble in ether.
Diclofenac sodium is hygroscopic.

Identification (1) Take 1 mL of a solution of Diclofenac Sodium in methanol (1 in 250), add 1 mL of nitric acid: a dark red color is observed.

(2) Perform the test with 5 mg of Diclofenac Sodium as directed under the Flame Coloration Test (2): a light green color is observed.

(3) Determine the infrared spectra of Diclofenac Sodium and Diclofenac Sodium RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Diclofenac Sodium (1 in 100) responds to the Qualitative Tests for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Diclofenac Sodium in 20 mL of methanol: the solution is colorless to pale yellow. Determine the absorbance of this solution at 440 nm as directed under Ultraviolet-visible Spectrophotometry: not more than 0.050.

(2) ***Heavy metals***—Proceed with 2.0 g of Diclofenac Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Diclofenac Sodium according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Diclofenac Sodium in 50 mL of the mobile phase and use this solution as the test solution. Pipet 2.0 mL of the test solution and add the mobile phase to make exactly 50 mL. Pipet 5.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography under the following operating conditions. Measure each peak area of these solutions by the automatic integration method: the area of peak other than the peak of diclofenac from the test solution is not larger than that of the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4.6mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (3 in 2500) (4 : 3).

Flow rate: Adjust the flow rate so that the retention time of diclofenac is about 20 minutes.

System suitability

System performance: Dissolve 35 mg of ethyl parahydroxybenzoate and 50 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. Take 1 mL of this solution, add the mobile phase to make exactly 50 mL. When the procedure is run with 20 μL of this solution, as directed under the above operating conditions, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 5.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of peak area of Diclofenac is not more than 2.0 %.

Time span of measurement: About twice as long as the retention time of diclofenac beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

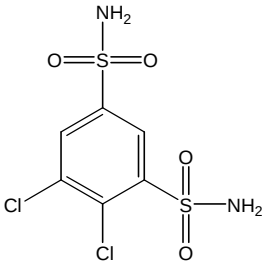
Assay Weigh accurately about 0.5 g of Diclofenac Sodium, previously dried, dissolve in 40 mL of water in a separatory funnel, add 2.0 mL of dilute hydrochloric acid and extract with 50 mL of chloroform. Extract again with two 20 mL volumes of chloroform and filter the extract each time through a pledget of absorbent cotton, moistened with chloroform. Wash the tip of the separatory funnel and the absorbent cotton, with 15 mL of chloroform, combine the washing with the extracts,

add 10.0 mL of a solution of 1 mol/L hydrochloric acid TS in ethanol (99.5) (1 in 100) and titrate with 0.1 mol/L potassium hydroxide-ethanol VS from the first equivalent point to the second equivalent point (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 31.813 mg of $C_{14}H_{10}Cl_2NNaO_2$

Containers and Storage ***Containers***—Tight containers.

Diclofenamide



$C_6H_6Cl_2N_2O_4S_2$: 305.16

4,5-Dichlorobenzene-1,3-disulfonamide [*120-97-8*]

Diclofenamide, when dried, contains not less than 98.0 % and not more than 101.0 % of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$).

Description Diclofenamide is a white, crystalline powder.
Diclofenamide is very soluble in *N,N*-dimethylformamide, soluble in ethanol (95), slightly soluble in ether and very slightly soluble in water.
Diclofenamide dissolves in sodium hydroxide TS.

Identification (1) Dissolve 10 mg each of Diclofenamide and Diclofenamide RS in 100 mL each of 0.01 mol/L sodium hydroxide TS. To 10 mL each of these solutions, add 0.1 mL each of hydrochloric acid and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Diclofenamide and Diclofenamide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 237 ~ 240 °C.

Purity (1) ***Chloride***—Dissolve 0.10 g of Diclofenamide in 10 mL of *N,N*-dimethylformamide and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS, add 10 mL of *N,N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.160 %).

(2) ***Selenium***—Take 0.10 g of Diclofenamide, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1 : 1) and 2 mL of nitric acid and heat in a water-batch until no more brown gas evolves and the solution becomes to be a pale yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL and use this solution as the test solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1 : 1) and 6 mL of nitric acid, then add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Specctrophotometry according to the following conditions and determine constant absorbance, A_T and A_S, obtained on a recorder after rapid increasing of the absorption: A_T is smaller than A_S (not more than 30 ppm). Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp
Wavelength: 196.0 nm
Temperature of sample atomizer: When an electric Furnace is used, about 1000 °C
Carrier gas: Nitrogen and argon

(3) ***Heavy metals***—Proceed with 2.0 g of Diclofenamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Diclofenamide in 50 mL of the mobile phase and use this solution as the test solution. Pipet 2.0 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of diclofenamide from the test solution is not larger than the peak area of diclofenamide from the standard solution.

Operating conditions
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions under Assay.
System suitability
Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of diclofenamide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5 % of that of diclofenamide obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diclofenamide is not more than 1.0 %.

Time span of measurement: About 5 times as long as the retention time of diclofenamide.

Loss on Drying Not more than 1.0 % (1 g, at a pressure not exceeding 0.67 kPa, 100 °C, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Diclofenamide and Diclofenamide RS, previously dried and dissolve in 30 mL each of the mobile phase. To each solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S of the peak area of diclofenamide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of diclofenamide } (C_6H_6Cl_2N_2O_4S_2) = \text{Amount (mg) of Diclofenamide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (3 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of sodium phosphate TS and acetonitrile (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of diclofenamide is about 7 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, diclofenamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diclofenamide to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Diclofenamide Tablets

Diclofenamide Tablets contain not less than 92.0 % and not more than 108.0 % of the labeled amount of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$: 305.16).

Method of Preparation Prepare as directed under Tablets, with Diclofenamide.

Identification (1) Weigh a portion of Diclofenamide Tablets, previously powdered, equivalent to 0.2 g of Diclofenamide according to the labeled amount, add 20 mL of methanol, shake and filter. Evaporate the filtrate on a water-bath to dryness. Dissolve 10 mg of the residue in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of this solution, add 0.1 mL of hydrochloric acid and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 284 nm and 288 nm and between 293 nm and 297 nm.

Dissolution Test Take 1 tablet of Diclofenamide Tablets and perform the test using 900 mL of water as a dissolution solution at 50 revolutions per minute as directed in the Method 2 under the Dissolution Test. After 60 minutes from the start of the test, take 20 mL or more of the dissolved solution and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL so that each mL contains about 55 μg of diclofenamide, and use this solution as the test solution. Separately, weigh accurately about 55 mg of Diclofenamide RS, previously dried at a pressure not exceeding 0.67 kPa at 100 °C for 5 hours, dissolve in 10 mL of ethanol (95) and add water to make exactly 100 mL. Pipet 10.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Measure the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 285 nm as directed under Ultraviolet-visible Spectrophotometry.

The dissolution rate of Diclofenamide Tablets in 60 minutes is not less than 70.0 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of diclofenamide } (C_6H_6Cl_2N_2O_4S_2) = W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90$$

W_S: Amount (mg) of Diclofenamide RS,

C: Labeled amount (mg) of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$) in 1 tablet.

Uniformity of Dosage Units It meets the require-

ment.

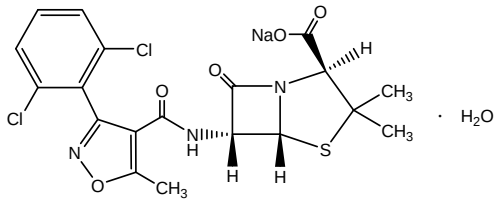
Assay Weigh accurately and powder not less than 20 tablets of Diclofenamide Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$), add exactly 25 mL of the mobile phase and shake for 15 minutes and centrifuge. Pipet 10.0 mL of the supernatant liquid, add 4.0 mL of the internal standard solution and the mobile phase to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Diclofenamide RS, previously dried at 100 °C at a pressure not exceeding 0.67 kPa for 5 hours, dissolve in 30 mL of the mobile phase, add 10.0 mL of the internal standard solution and the mobile phase to make exactly 50 mL and use this solution as the standard solution. Proceed as directed in the Assay under Diclofenamide.

$$\text{Amount (mg) of diclofenamide } (C_6Cl_2N_2O_4S_2) = \text{Amount (mg) of Diclofenamide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (3 in 5000).

Containers and Storage ***Containers***—Well-closed containers.

Dicloxacillin Sodium Hydrate



$C_{19}H_{16}Cl_2N_3NaO_5S \cdot H_2O$: 510.32

Sodium(2*S*,5*R*,6*R*)-6-[[3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrate [*13412-64-1*]

Dicloxacillin Sodium Hydrate contains not less than 910 μg (potency) and not more than 1020 μg (potency) per mg of dicloxacillin ($C_{19}H_{16}Cl_2N_3NaO_5S$: 470.33), calculated on the anhydrous basis

Description Dicloxacillin Sodium Hydrate is a white to light yellowish white crystalline powder.
Dicloxacillin Sodium Hydrate is freely soluble in water and in methanol, and soluble in ethanol (95).

Identification (1) Determine the absorption spectra of the solutions of Dicloxacillin Sodium Hydrate and Dicloxacillin Sodium RS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dicloxacillin Sodium Hydrate and Dicloxacillin Sodium RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The solution of Dicloxacillin Sodium Hydrate responds to the Qualitative test 1) for sodium salt.

Crystallinity It meets the requirements.

pH The pH of a solution obtained by dissolving 0.1 g of Dicloxacillin Sodium Hydrate in 10 mL of water is between 4.5 and 7.5.

Purity ***Dimethylaniline***—Weigh accurately about 1.0 g of Dicloxacillin Sodium Hydrate, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid and add water to make 50 mL. Pipet 5.0 mL of this solution and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS, add 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and the standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Purity (\%) of dimethylaniline}}{\text{Amount (mg) of Dicloxacillin Sodium Hydrate taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. Pipet 5.0 mL of this solution, add cyclohexane to make 100 mL and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 2 mm in internal diameter and about 2 m in length, packed with diatomaceous earth coated with 50 % phenyl to 50 % methylpolysiloxane for gas chromatography equivalent to 3 % of the mass.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water 3.0 ~ 4.5 % (0.1 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Dicloxacillin Sodium Hydrate is used in a sterile preparation.

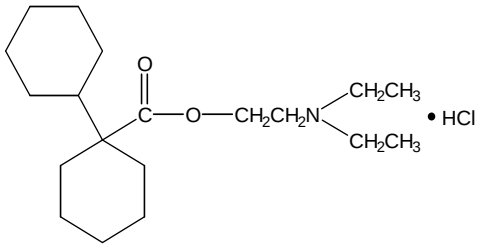
Pyrogen Test It meets the requirement, when Dicloxacillin Sodium Hydrate is used in a sterile preparation. Weigh appropriate amount of Dicloxacillin Sodium Hydrate, dissolve in Isotonic Sodium Chloride Injection, make the solution so that each mL contains 20 mg (potency), and use the solution as the test solution. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay Weigh accurately an amount of Dicloxacillin Sodium Hydrate, dissolve in 1 % phosphate buffer, pH 6.0 to make the solution containing 1 mg (potency) dicloxacillin per mL and use the solution as the test solution. Separately, weigh accurately an amount of Dicloxacillin Sodium Hydrate RS, dissolve 1 % phosphate buffer, pH 6.0 to make the solution so that each mL contains 1 mg (potency) and use the solution as the standard solution. Keep the standard solution at not exceeding 5 °C and use within 7 days. Pipet 1 mL of each of the test solution and the standard solution in iodine bottle, add exactly 2 mL of 1 mol/L sodium hydroxide TS, stand for 15 minutes, add 2 mL of diluted hydrochloric acid (1 in 10) and 10.0 mL of 0.01 mol/L iodine solution, stand for 15 minutes, if necessary, add 5 mL of carbon tetrachloride , mix with shaking, titrate the test solution and the standard solution with 0.01 mol/L sodium thiosulfate by using microburet (indicator: if necessary, 0.2 ~ 0.5 mL of starch TS), and determine the consumed volume (mL) of 0.01 mol/L of iodine solution, V_T and V_S. Separately, add exactly 10 mL of 0.01 mol/L iodine solution to the test solution and the standard solution, perform a blank determination without 15 minutes standing and make any necessary correction.

$$\text{Amount [μg (potency)] of dicloxacillin sodium } (C_{19}H_{16}Cl_2N_3NaO_5S) = \text{Amount [μg (potency)] of Dicloxacillin Sodium Hydrate RS} \times \frac{V_T}{V_S}$$

Containers and Storage ***Containers***—Tight containers.

Dicyclomine Hydrochloride



$C_{19}H_{35}NO_2 \cdot HCl$: 345.95

2-(Diethylamino)ethyl-1-cyclohexylcyclohexane-1-carboxylate hydrochloride [*67-92-5*]

Dicyclomine Hydrochloride contains not less than 99.0 % and not more than 102.0 % of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$), calculated on the anhydrous basis.

Description Dicyclomine Hydrochloride is a white fine crystalline powder, is odorless and has a bitter taste.
Dicyclomine Hydrochloride is freely soluble in ethanol (95) or in chloroform, soluble in water and very slightly soluble in ether.

Identification (1) Take 5 mL of a solution of Dicyclomine Hydrochloride (1 in 500) and add 2 mL of 2 mol/L nitric acid TS and 2 mL of silver nitrate TS: a white precipitate is formed. The precipitate does not dissolve in nitric acid and dissolves excessive amount of ammonia TS.

(2) Determine the infrared spectra of Dicyclomine Hydrochloride and Dicyclomine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavenumbers.

Melting Point 169 ~ 174 °C.

pH Dissolve 1.0 g of Dicyclomine Hydrochloride in 100 mL of water: the pH of this solution is between 5.0 and 5.5.

Purify ***Sulfate***— Perform the test with 0.5 g of Dicyclomine Hydrochloride and use this solution as the test solution. The color of the test solution is not stronger than that of the control solution D.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

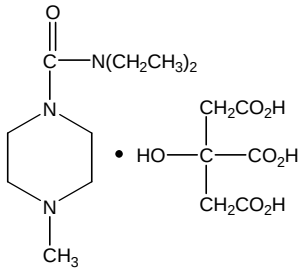
Assay Weigh accurately about 0.6 g of Dicyclomine Hydrochloride, dissolve in 70 mL of acetic acid (100), add 10 mL of mercuric acetate TS and titrate with 0.1 mol/L perchloric acid VS until blue color develops (indicator: 1 drop of methylosanyl chloride TS). Per-

form a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.595 mg of $C_{19}H_{35}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Diethylcarbamazine Citrate



$C_{10}H_{21}N_3O \cdot C_6H_8O_7$: 391.42

N,N-Diethyl-4-methylpiperazine-1-carboxamide; 2-hydroxypropane-1,2,3-tricarboxylic acid [*1642-54-2*]

Diethylcarbamazine Citrate, when dried, contains not less than 98.0 % and not more than 101.0 % of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$).

Description Diethylcarbamazine Citrate is a white, crystalline powder, is odorless and has an acid and bitter taste.
Diethylcarbamazine Citrate is very soluble in water, soluble in ethanol (95) and practically insoluble in acetone, in chloroform or in ether.
A solution of Diethylcarbamazine Citrate (1 in 20) is acidic.
Diethylcarbamazine Citrate is hygroscopic.

Identification (1) Dissolve 0.5 g of Diethylcarbamazine Citrate in 2 mL of water, add 10 mL of sodium hydroxide TS and extract with four 5 mL volumes of chloroform. Wash the combined chloroform extracts with 10 mL of water and evaporate chloroform on a water-bath and add 1 mL of ethyl iodide to the residue and boil gently with the aid of a reflux condenser for 5 minutes. Evaporate excess amount of ethyl iodide through air and add 4 mL of ethanol (95). Cool the ethanol solution in an ice-bath with continuous stirring, add ether until precipitates are produced and stir until crystallization is evident. Allow to stand in the ice-bath for 30 minutes and filter and collect the precipitate. Dissolve the precipitate in 4 mL of ethanol (95), repeat the recrystallization in the same manner and then dry at 105 °C for 4 hours: the melting point is between 151 °C and 155 °C.

(2) Neutralize the residue after extracting with chloroform in (1) with dilute hydrochloric acid: the solution responds to the Qualitative Tests (2) and (3) for citrate.

Melting Point 135.5 ~ 138.5 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Diethylcarbamazine Citrate according to Method 4 and perform the test. Prepare the control solution with 4.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately 300 mg of Diethylcarbamizine Citrate, add 100 mL of phosphate buffer and mix. Filter or centrifuge and use the filtrate or supernatant as the test solution. Separately, weigh accurately a suitable amount of Diethylcarbamazine Citrate RS, add phosphate buffer to make a solution containing about 3 μg per mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography under the following operating conditions. Determine A_i, the area of each peak other than the principal peak from the test solution, and A_S, the peak area of diethylcarbamizine in the standard solution, and calculate the content of each related substance in the test solution according to the following equation: not more than 0.1 %.

$$\text{Content (\%) of each related substance} = 10000 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

C: Concentration (mg/mL) of Diethylcarbamazine Citrate RS in the standard solution
W: Amount (mg) of Diethylcarbamizine Citrate taken

Phosphate buffer—Dissolve 31.24 g of potassium dihydrogen phosphate in 1000 mL of water.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength 220 nm)
Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)
Mobile phase: A mixture of a 1 % aqueous solution of potassium dihydrogen phosphate and methanol (900:100)
Flow rate: 0.8 mL/minute
System suitability
System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, the relative standard deviation of the area of the principal peak is not more than 2.0 %.

Loss on Drying Not more than 1.0 % (2 g, 105 °C, 4 hours).

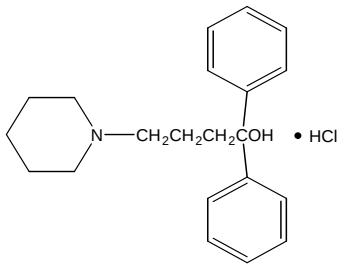
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.75 g of Diethylcarbamazine Citrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.142 mg of $C_{10}H_{21}N_3O \cdot C_6H_8O_7$

Containers and Storage ***Containers***—Tight containers.

Difenidol Hydrochloride



$C_{21}H_{27}NO \cdot HCl$: 345.91

1,1-Diphenyl-4-piperidin-1-ylbutan-1-ol hydrochloride [*3254-89-5*]

Difenidol Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of difenidol hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

Description Difenidol Hydrochloride appears as white crystals or crystalline powder and is odorless.
Difenidol Hydrochloride is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in water or in acetic acid (100) and practically insoluble in ether.

Melting point—About 217 °C (with decomposition).

Identification (1) Dissolve 10 mg of Difenidol Hydrochloride in 1 mL of sulfuric acid: an orange-red color is observed. Take this solution, add carefully 3 drops of water: the solution becomes yellowish brown and colorless on the addition of 10 mL of water.

(2) Take 5 mL of a solution of Difenidol Hydrochloride (1 in 100) and add 2 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Take 10 mL of a solution of Difenidol Hydrochloride (1 in 100) and add 2 mL of sodium hydroxide TS and extract with two 15 mL volumes of chloroform. Combine the extracts, wash with three 10 mL volumes of water, evaporate the chloroform on a water-bath and dry the residue in a desiccator (in vacuum, silica gel, 55 °C) for 5 hours: the residue melts between 103 °C and 106 °C.

(4) A solution of Difenidol Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Difenidol Hydrochloride in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.7 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Difenidol Hydrochloride in 10 mL of methanol: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Difenidol Hydrochloride according to Method 2 and perform the test (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Difenidol Hydrochloride according to Method 3 and perform the test (not more than 1 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Difenidol Hydrochloride in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 10 mg of 1,1-diphenyl-4-piperidino-1-butene hydrochloride RS in methanol to make exactly 20 mL, pipet 1.0 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the test solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, methanol and acetic acid (100) (10 : 2 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 5 hours).

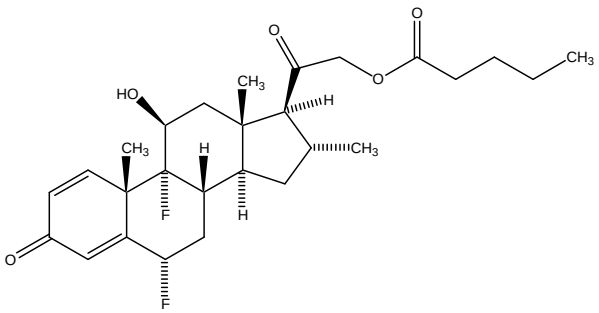
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.35 g of Difenidol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100) by warming, if necessary, cool, add 30 mL of acetic anhydride and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.295 mg of $C_{21}H_{27}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Diflucortolone Valerate



$C_{27}H_{36}F_2O_5$: 478.57

[2-[(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*S*)-6,9-Difluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-7,8,11,12,14,15,16,17-octahydro-6*H*-cyclopenta[a]phenanthren-17-yl]-2-oxoethyl]pentanoate
[*59198-70-8*]

Diflucortolone Valerate contains not less than 97.0 % and not more than 102.0 % of diflucortolone valerate ($C_{27}H_{36}F_2O_5$), calculated on the dried basis.

Description Diflucortolone Valerate is a white crystalline powder.
Diflucortolone Valerate is freely soluble in dichloromethane or in 1,4-dioxane, sparingly soluble in ether, slightly soluble in methanol, and practically insoluble in water.

Identification Determine the absorption spectra of solutions of Diflucortolone Valerate and Diflucortolone Valerate RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: +110 ~ +115° (0.1 g calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Diflucortolone Valerate according to Method 2 under Heavy Metals Limit Test and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Perform the test with 10 μL of the test solution from the Assay as directed under Liquid Chromatography. Determine each peak area of the test solution by the automatic integration method and calculate the amount of each peak by the area percentage method: the amount of each peak of flucortolone valerate, 12α-diflucortolone valerate and *Δ*4-diflucortolone valerate is not more than 0.6 %, and the amount of clocortolone is not more than 0.3 %. The amount of each peak other than those mentioned above is not more than 0.1 %, and the sum of the amounts of related substances other than diflucortolone valerate is not more than 2.0 %.

Operating conditions
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.
System suitability
System performance and system repeatability: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Pipet 0.1 mL of the test solution, add a mixture of water and acetonitrile (1:1) to make exactly 10 mL and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution and add a mixture of water and accetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of diflucortolone valerate obtained from 10 μL of this solution is equivalent to 3.5 % to 6.5 % of the peak area of diflucortolone valerate from the system suitability solution.
Relative retention time: The relative retention times of flucortolone valerate, 12α-diflucortolone valerate, *Δ*4-diflucortolone valerate and clocortolone valerate, with respect to the diflucortolone valerate peak, are 0.97, 1.03, 1.05 and 1.09, respectively.
Time span of measurement: About 1.4 times as long as the retention time of diflucortolone valerate, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1.0 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 5 mg each of Diflucortolone Valerate and Diflucortolone Valerate RS (determine the loss on drying in the same conditions as Diflucortolone Valerate), dissolve each in a mixture of water and acetonitrile (1:1) to make exactly 10 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peak for diflucortolone valerate in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of diflucortolone valerate } (C_{27}H_{36}F_2O_5) = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Diflucortolone Valerate, calculated on the dried basis

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 238 nm)
Column: A stainless steel column 4.6 in internal diameter and 25 cm in length, packed with sulfonamide

group bound to hexadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, adjusted to pH 3.0 with phosphoric acid, and acetonitrile (11:9)

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-10	100→90	0→10
10-25	90	10
25-45	90→35	10→65
45-50	35	65

Flow rate: 1.0 mL/minute

System suitability

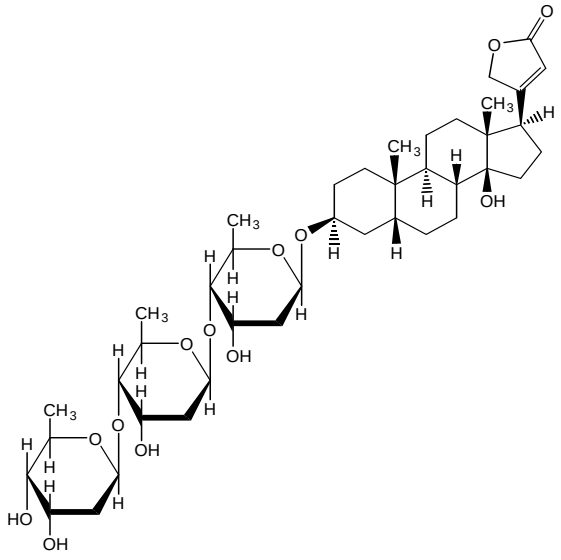
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of diflucortolone valerate is not less than 10000 with the symmetry factor being not more than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diflucortolone valerate is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Digitoxin



$C_{41}H_{64}O_{13}$: 764.94

4-[(3*S*,5*R*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-3-[(2*S*,4*S*,5*R*,6*R*)-5-[(2*S*,4*S*,5*R*,6*R*)-5-[(2*S*,4*S*,5*R*,6*R*)-4,5-Dihydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-14-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-5*H*-furan-2-one [*71-63-6*]

Digitoxin, when dried, contains not less than 90.0 % and not more than 101.0 % of digitoxin ($C_{41}H_{64}O_{13}$).

Description Digitoxin is a white to pale yellow, crystalline powder and is odorless.

Digitoxin is soluble in chloroform, sparingly soluble in methanol or in ethanol (95) and practically insoluble in water or in ether.

Identification (1) Transfer 1 mg of Digitoxin to a small test tube about 10 mm in internal diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10000) and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring free from a reddish color is observed and the color of the upper layer near the contact zone changes to green through purple. Finally the color of the entire acetic acid layer changes to green through deep blue.

(2) Take 2 mg of Digitoxin, add 25 mL of a freshly prepared solution of *m*-dinitrobenzene in ethanol (95) (1 in 100) and dissolve by shaking. Pipet 2 mL of this solution, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200) and mix: a red-purple color is observed slowly and then fades.

(3) Dissolve 1 mg each of Digitoxin and Digitoxin RS in a mixture of ethanol (95) and chloroform (1 : 1) to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84 : 15 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate and heat at 110 °C for 10 minutes: the spot from the test solution shows the same R_f value as the spot from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: + 16 ~ + 18° (after drying, 0.50 g, chloroform, 20 mL, 200 mm).

Purity ***Digitonin***—Dissolve 10 mg of Digitoxin in 2 mL of ethanol (95) in a test tube having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently and allow to stand for 10 minutes: no turbidity is pro-

duced.

Loss on Drying Not more than 1.5 % (0.5 g, in vacuum, 100 °C, 2 hours).

Residue on Ignition Not more than 0.5 % (0.1 g).

Assay Dissolve about 20 mg each of Digitoxin and Digitoxin RS, previously dried and accurately weighed, in methanol to make exactly 200 mL. Pipet 5.0 mL each of these solutions, add 10.0 mL of the internal standard solution to each solution, add 12.5 mL of water, then add methanol to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S of the peak area of digitoxin to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of digitoxin } (C_{41}H_{64}O_{13}) = \text{Amount (mg) of Digitoxin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acenaphthene in methanol (3 in 1000000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm to 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of methanol and water (3 : 1).

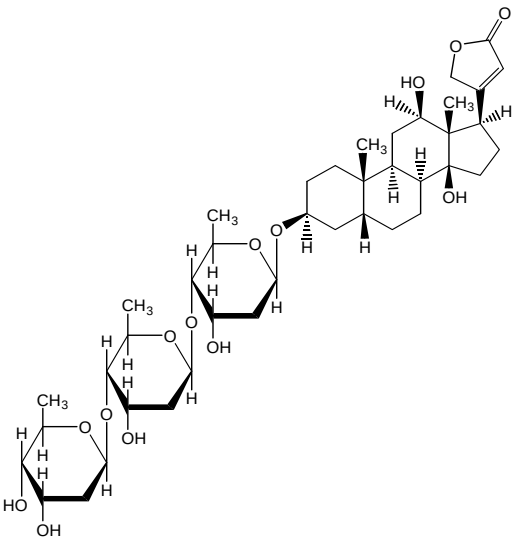
Flow rate: Adjust the flow rate so that the retention time of digitoxin is about 5 minutes.

Selection of column: Proceed with 50 μL of the standard solution according to the above conditions, use a column givion elution of digitoxin and the internal standard in this order with a resolution between their peaks being not less than 6.0.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Digoxin



$C_{41}H_{64}O_{14}$: 780.94

4-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*S*)-3-[(2*S*,4*S*,5*R*,6*R*)-5-[(2*S*,4*S*,5*R*,6*R*)-5-[(2*S*,4*S*,5*R*,6*R*)-4,5-Dihydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-5*H*-furan-2-one [*20830-75-5*]

Digoxin, when dried, contains not less than 96.0 % and not more than 106.0 % of digoxin ($C_{41}H_{64}O_{14}$).

Description Digoxin appears as colorless to white crystals or white, crystalline powder.

Digoxin is freely soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in acetic acid (100) and practically insoluble in water.

Identification (1) Transfer 1 mg of Digoxin to a small test tube, about 10 mm in internal diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10000) and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring free from a reddish color is observed and the color of the upper layer near the contact zone changes to green through purple. Finally the color of the entire acetic acid layer shows a green color through a deep blue color.

(2) Determine the infrared spectra of Digoxin and Digoxin RS, both previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: + 10.0 ~ + 13.0° (after drying, 0.2 g, pyridine, 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Digoxin in 15 mL of diluted ethanol (4 in 5) by warming: the solution is clear and colorless.
(2) ***Related substances***—Weigh accurately about 25.0 mg of Digoxin, dissolve in 50 mL of warm ethanol, cool, add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water, add dilute ethanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately 5.0 mg of Gitoxin RS, previously dried under reduced pressure at 105 ºC for 1 hour, and add a mixture of acetonitrile and water (7 : 3) to make exactly 200 mL. Pipet accurately 2 mL of this solution, add dilute ethanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution according to the following condition as directed under Liquid Chromatography and determine the peak are of gitoxin in the test (A_T) and the standard (A_S) solutions. A_T is not larger than A_S, and the total of the areas of the peaks other than digoxin and gitoxin, obtained by the area percentage method, is not more than 3 %.

Operating conditions
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.
System suitability
Test for required detectability: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol, cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make 50 mL and use this solution as the system suitability solution. Pipet 2 mL of the system suitability solution, add dilute ethanol to make exactly 100 mL, pipet 5 mL of this solution and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained form 10 μL of this solution is equivalent to 0.07 % to 0.13 % of that from the system suitability solution.
System performance: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol, cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl *p*-hydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5 %.
Time span of measurement: About 4 times as long as the retention time of digoxin after the solvent peak

Loss on Drying Not more than 1.0 % (0.5 g, in vacuum, 105 °C, 1 hours).

Residue on Ignition Not more than 0.5 % (0.1 g).

Assay Weigh accurately about 25 mg each of Digoxin and Digoxin RS, previously dried, dissolve in 50 mL each of warm ethanol, cool and add ethanol (95) to make exactly 100 mL each. Pipet accurately 10 mL each of these solutions, add 5 mL each of the internal standard solution, add 10 mL each of water and dilute ethanol to make exactly 50 mL each, and use these solutions as the test and standard solutions, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions and determine the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard for the test and standard solutions, respectively.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = \text{Amount (mg) of Digoxin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in ethanol (95) (1 in 4000)

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 220 nm)
Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)
Column temperature: A constant temperature of about 30 °C.
Mobile phase: A mixture of water and acetonitrile (7:3).
Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.
System suitability
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Digoxin Injection

Digoxin Injection is an aqueous solution for injection. Digoxin Injection contains not less than 90.0 % and not more than 105.0 % of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$: 780.94).

Method of Preparation Prepare as directed under Injections, with a solution of Digoxin in 10 to 50 vol % ethanol.

Description Digoxin Injection is a clear, colorless liquid.

Identification Dilute Digoxin Injection, if necessary, with ethanol so that each mL contains about 2.5 mg of Digoxin according to the labeled amount and use this solution as the test solution. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7 : 3) to a distance of about 10 cm and air-dry the plate. Spray evenly the plate with a freshly prepared mixture containing one volume of sodium toluenesulfon chloroamide trihydrate solution (3 in 100) and four volumes of trichloroacetic acid in ethanol (95) (1 in 4), heat at 110 °C for 10 minutes and examine the plate under UV light (the principal wavelength of 366 nm). The R_f values of the principal spots obtained from the test solution and the standard solution are not different each other.

Purity ***Related substances***—Pipet a volume of Digoxin Injection, equivalent to 2.5 mg of digoxin according to the labeled amount, dissolve in 50 mL of dilute ethanol and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography under the following operating conditions. Determine each peak area from the test solution by the automatic integration method and calculate their amount by the area percentage method: the sum of the related substance peaks with respect to the main peak is not more than 5 %.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions under the Assay.

System suitability

Test for required detectability: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make 50 mL and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, add dilute ethanol to make exactly 100 mL, pipet 5 mL of this solution and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained form 10 μL of this solution is equivalent to 0.07 % to 0.13 % of that from the solution for system suitability test.

System performance: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl *p*-hydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5 %.

Time span of measurement: About 4 times as long as the retention time of digoxin after the solvent peak

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 200 EU/mg of Digoxin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Alcohol Number 0.8 ~ 1.2 (Method 1).

Assay Transfer an exactly measured volume of Digoxin Injection, equivalent to 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), add 5 mL of the internal standard solution and dilute ethanol to make 50 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105 °C for 1 hour, dissolve in 50 mL of warm ethanol, cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard, obtained with the test solution and the standard solution, respectively.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14})$$

$$= \text{Amount (mg) of Digoxin RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution–A solution of propyl *p*-hydroxybenzoate in ethanol (95) (1in 40000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.5 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μ m in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Digoxin Tablets

Digoxin Tablets contain not less than 90.0 % and not more than 105.0 % of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$: 780.94).

Method of Preparation Prepare as directed under Tablets, with Digoxin.

Identification Weigh a portion of powdered Digitoxin Tablets, equivalent to 0.5 mg of digoxin ($C_{41}H_{64}O_{14}$) according to the labeled amount, add 2 mL of methanol, shake for 10 mL to mix, filter and use the filtrate as the test solution. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7 : 3) to a distance of about 10 cm and air-dry the plate. Spray evenly the plate with a freshly prepared mixture containing one volume of sodium toluenesulfon chloroamide trihydrate solution (3 in 100) and four volumes of trichloroacetic acid in ethanol (95) (1 in 4), heat at 110 °C for 10 minutes and examine the plate under UV light (the principal wavelength of 366 nm). The R_f values of the principal spots obtained from the test solution and the standard solution are not different each other.

Purity ***Related substances***—Weigh and powder not less than 20 Digoxin Tablets. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of digoxin, add 30 mL of dilute ethanol, sonicate for 20 minutes and shake for 5 minutes. Allow to cool, add dilute ethanol to make 50 mL, filter and use the filtrate as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography under the following operating conditions. Determine each peak area from the test solution by the automatic integration method and calculate their amount by the area percentage method: the sum of the related substance peaks with respect to the main peak is not more than 5 %.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions under the Assay.

System suitability

Test for required detectability: Dissolve 25 mg of Digoxin Tablets in 50 mL of warm ethanol, cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make 50 mL and use this solution as the system suitability solution. Pipet 2 mL of the system suitability solution, add dilute ethanol to make exactly 100 mL, pipet 5 mL of this solution and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained form 10 μL of this solution is equivalent to 0.07 % to 0.13 % of that from the system suitability solution.

System performance: Dissolve 25 mg of Digoxin Tablets in 50 mL of warm ethanol, cool and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl *p*-hydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5 %.

Time span of measurement: About 4 times as long as the retention time of digoxin after the solvent peak

Dissolution Test Take 1 tablet of Digoxin Tablets

and perform the test using 500 mL of diluted hydrochloric acid (3 in 500) as dissolution solution, at 100 revolutions per minute, as directed in the Method 1 under the Dissolution Test. After 60 minutes from the start of the test, take 30 mL or more of the dissolved solution and filter through a membrane filter (less than 0.8 μm in pore size). Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 25 mg of digoxin RS, previously dried in vacuum at 105 °C for 1 hour, dissolve in a small volume of ethanol (95) and add a mixture, containing four volumes of ethanol (95) and one volume of water, to make exactly 500 mL. Pipet 5.0 mL of this solution, add the test solution to make exactly 500 mL and use this solution as the standard solution**.** Pipet 2.0 mL each of the test solution, the standard solution, the dissolution solution and transfer to brown glass-stoppered test tubes, T, S and B, respectively**.** Add exactly 10 mL of 0.12 mg/mL ascorbic acid-hydrochloric acid TS to each of these solutions and shake**.** Immediately add 1.0 mL of dilute hydrogen peroxide TS, shake well and allow to stand at a constant temperature between 25 °C and 30 °C for 45 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of the test solution, standard solution and the dissolution solution, respectively, at about 360 nm of the excitation wavelength and at 485 nm of the fluorescence wavelength as directed under the Fluorometry.
The dissolution rate of Digoxin Tablets in 60 minutes is not less than 65.0 %.
No retest requirement is applied to Digoxin Tablets.

$$\text{Dissolution rate (\%) with respect to the labeled amount of digoxin } (C_{41}H_{64}O_{14}) = W_S \times \frac{F_T - F_B}{F_S - F_B} \times \frac{1}{C}$$

W_S: Amount (mg) of Digoxin RS
C: Labeled amount (mg) of digoxin ($C_{41}H_{64}O_{14}$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.
To 1 tablet of Digoxin Tablets, add 0.5 mL of water to disintegrate, add exactly 0.5 mL of the internal standard solution, and add V mL of dilute ethanol to render the concentration to be about 21 μg of digoxin ($C_{41}H_{64}O_{14}$) in 1 mL. Exposure this solution to ultrasonic waves, shake for 5 minutes to mix, filter, and use the filtrate as the test solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105 °C for 1 hour, dissolve in 50 mL of warm ethanol , cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution and add ethanol (95) to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 0.5 mL of the internal standard solution, add 1.5 mL of water and (V-2) mL of dilute ethanol and use this as the standard solution. Perform the test with the test solution and standard solution as directed in the Assay.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = \text{Amount (mg) of Digoxin RS} \times \frac{Q_T}{Q_S} \times \frac{1}{200}$$

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in ethanol (95) (1in 40000/V).

Assay Weigh accurately and powder not less than 20 Digoxin Tablets. Transfer an accurately weigh a portion of the powder, equivalent to about 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), add 30 mL of dilute ethanol, exposure to ultrasonic waves for 20 minutes and shake for 5 minutes to mix. Add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL. Centrifuge the solution and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105 °C for 1 hour, dissolve in 50 mL of warm ethanol, cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the internal standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard, obtained with the test solution and the standard solution, respectively.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = \text{Amount (mg) of Digoxin RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in ethanol (95) (1in 40000).

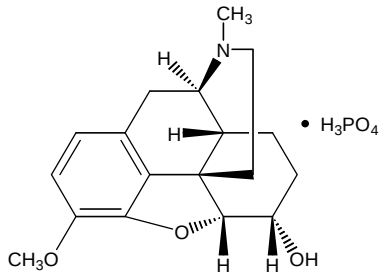
Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.5 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μ m in particle diameter).
Column temperature: A constant temperature of about 30 °C.
Mobile phase: A mixture of water and acetonitrile (7:3).
Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.
System suitability
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dihydrocodeine Phosphate



Hydrocodeine Phosphate $C_{18}N_{23}NO_3 \cdot H_3PO_4$: 399.38

4,5-alpha-Epoxy-3-methoxy-17-methyl-morphinan-6-ol [*24204-13-5*]

Dihydrocodeine Phosphate contains not less than 98.0 % and not more than 101.0 % of dihydrocodeine phosphate ($C_{18}N_{23}NO_3 \cdot H_3PO_4$), calculated on the dried basis.

Description Dihydrocodeine Phosphate is a white to yellowish white, crystalline powder.
Dihydrocodeine Phosphate is freely soluble in water or in acetic acid (100), slightly soluble in ethanol and practically insoluble in ether.
When dissolve 1.0 g of Dihydrocodeine Phosphate in 10 mL of water, the pH of this solution is between 3.0 and 5.0.
Dihydrocodeine Phosphate is affected by light.

Identification (1) Determine the absorption spectra of solutions of Dihydrocodeine Phosphate and Dihydrocodeine Phosphate RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dihydrocodeine Phosphate and Dihydrocodeine Phosphate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Dihydrocodeine Phosphate (1 in 20) responds to the Qualitative Tests (1) for phosphate.

Purity (1) ***Chloride***—Perform the test with 0.5 g of Dihydrocodeine Phosphate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.021 %).

(2) ***Sulfat***e—Perform the test with 0.20 g of Dihydrocodeine Phosphate. Prepare the control solution with 1.0 mL of 5 mol/L sulfuric acid (not more than 0.240 %).

(3) ***Related substances***—Dissolve 0.20 g of Dihydrocodeine Phosphate in 10 mL of diluted ethanol (1 in 2) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted ethanol (1 in 2) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 10 μL of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated ethanol, toluene, acetone and ammonia solution (28) (14 : 14 : 7 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 4 hours).

Assay Weigh accurately about 0.5 g of Dihydrocodeine Phosphate, dissolve in 70 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.938 mg of $C_{18}N_{23}NO_3 \cdot H_3PO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

1 % Dihydrocodeine Phosphate Powder

1 % Hydrocodeine Phosphate Powder

1 % Dihydrocodeine Phosphate Powder contains not less than 0.90 % and not more than 1.10 % of dihydrocodeine phosphate ($C_{18}N_{23}NO_3 \cdot H_3PO_4$: 399.38).

Method of Preparation

Dihydrocodeine Phosphate	100 g
Lactose Hydrate	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1 % Dihydrocodeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 281 nm and 285 nm.

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately about 5 g of 1 % Dihydrocodeine Phosphate Powder and dissolve in water to make exactly 100 mL. Pipet 10.0 mL of this solution, add 10.0 mL of the internal standard solution and use this solution as the test solution. Separately, weigh accurately about 0.05 g of Dihydrocodeine Phosphate RS, separately determined its loss on drying (105 °C, 4 hours) and dissolve in water to make exactly 100 mL. Pipet 10.0 mL of this solution, add exactly 10 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard, for the test solution and the standard solution, respectively.

Amount (mg) of dihydrocodeine phosphate ($C_{18}N_{23}NO_3 \cdot H_3PO_4$) = Amount (mg) of Dihydrocodeine Phosphate RS, calculated on the dried basis $\times \dfrac{Q_T}{Q_S}$

Internal standard solution—A solution of Ethylefrine Hydrochloride (3 in 10000).

Operating conditions

Perform as directed in Assay under 10 % Dihydrocodeine Phosphate Powder.

Containers and Storage ***Containers***—Tight containers.

10 % Dihydrocodeine Phosphate Powder

10 % Hydrocodeine Phosphate Powder

10 % Dihydrocodeine Phosphate Powder contains not less than 9.3 % and not more than 10.7 % of dihydrocodeine phosphate ($C_{18}N_{23}NO_3 \cdot H_3PO_4$: 399.38).

Method of Preparation

Dihydrocodeine Phosphate	100 g
Lactose Hydarate	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10 % Dihydrocodeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 281 nm and 285 nm.

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately about 2.5 g of 10 % Dihydrocodeine Phosphate Powder and dissolve in water to make exactly 100 mL. Pipet 2.0 mL of this solution, add 10.0 mL of the internal standard solution and water to make 20 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Dihydrocodeine Phosphate RS, separately determined its loss on drying (105 ºC, 4 hours) and dissolve in water to make exactly 100 mL. Pipet 10.0 mL of this solution, add exactly 10 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard, for the test solution and the standard solution, respectively.

Amount (mg) of dihydrocodeine phosphate ($C_{18}N_{23}NO_3 \cdot H_3PO_4$) = Amount (mg) of Dihydrocodeine Phosphate RS, calculated on the dried basis $\times \dfrac{Q_T}{Q_S} \times 5$

Internal standard solution—A solution of Ethylefrine Hydrochloride (3 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000) and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution, add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

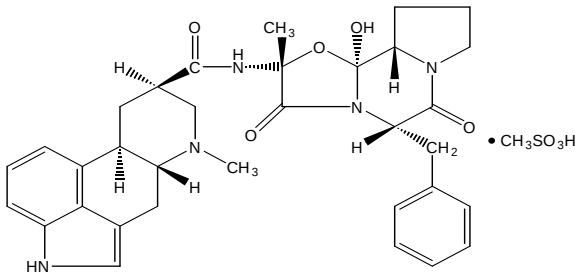
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Dihydroergotamine Mesilate



$C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$: 679.78

(2*R*,4*R*,7*R*)-*N*-[(1*S*,2*S*,4*R*,7*S*)-7-Benzyl-2-hydroxy-4-methyl-5,8-dioxo-3-oxa-6,9-diazatricyclo[7.3.0.02,6]dodecan-4-yl]-6-methyl-6,11-diazatetracyclo[7.6.1.02,7.012,16]hexadeca-1(16),9,12,14-tetraene-4-carboxamide; methanesulfonic acid [*6190-39-2*]

Dihydroergotamine Mesilate contains not less than 97.0 % and not more than 101.0 % of dihydroergotamine mesilate ($C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$), calculated on the dried basis.

Description Dihydroergotamine Mesilate is a white to yellowish white or grayish white to reddish white powder**.**

Dihydroergotamine Mesilate is freely soluble in acetic acid (100), sparingly soluble in methanol or in chloroform, slightly soluble in water or in ethanol (95) and practically insoluble in acetic anhydride or in ether.

Dihydroergotamine Mesilate is gradually affected by light.

Melting point—About 214 °C (with decomposition).

Identification (1) Dissolve 1 mg of Dihydroergotamine Mesilate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution, add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS and shake: a blue color is observed.

(2) Take 0.1 g of Dihydroergotamine Mesilate, add 0.4 g of sodium hydroxide, stir well and incinerate by gradual ignition. After cooling, add 10 mL of water to the residue, heat to boiling, cool and filter. To the filtrate, add 0.5 mL of hydrochloric acid: the solution responds to the Qualitative Tests for sulfate. Separately, to 0.1 g of Dihydroergotamine Mesilate, add 5 mL of dilute hydrochloric acid, shake for 5 minutes, filter and to the filtrate, add 1 mL of barium chloride TS: the solution is clear.

(3) Determine the absorption spectra of solutions of Dihydroergotamine Mesilate and Dihydroergotamine Mesilate RS in methanol (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Dihydroergotamine Mesilate and Dihydroergotamine Mesilate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -16.7 ~ -22.7° [0.5 g, when dried, a mixture of ethanol (99.5), chloroform and ammonia solution (28) (10 : 10 : 1), 20 mL, 100 mm].

pH Dissolve 50 mg of Dihydroergotamine Mesilate in 50 mL of water: the pH of this solution is between 4.4 and 5.4.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Dihydroergotamine Mesilate in 0.1 mL of a solution of methanesulfonic acid (7 in 100) and 50 mL of water: the solution is clear and has no more color than the following control solutions (1) or (2).

Control solution (1)—Pipet 0.60 mL of iron (III) chloride CS and 0.150 mL of cobalt (II) chloride CS, mix and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (2)—Pipet 0.60 mL of iron (III) chloride CS and 0.250 mL of cobalt (II) chloride CS and 0.1 mL of cupric sulfate CS, mix and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) ***Related substances***—Perform this procedure without exposure to daylight, using light-resistant vessels**.** Dissolve 0.10 g of Dihydroergotamine Mesilate in 5 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 200 mL and use this solution as the standard solution (1). Pipet 10.0 mL of the standard solution (1), add a mixture of chloroform and methanol (9 : 1) to make exactly 25 mL and use

this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethylacetate, methanol and ammonia solution (28) (50 : 50 : 6 : 1) to a distance of about 15 cm and dry the plate with cold wind within 1 minute**.** Develop the plate again immediately with a freshly prepared mixture of dichloromethane, ethylacetate, methanol and ammonia solution (28) (50 : 50 : 6 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate and dry the plate with warm wind: the spots other than the principal spot from the test solution are not more intense than that from the standard solution (1) and the spots, which are more intense than the spot from the standard solution (2), are not more than two.

Loss on Drying Not more than 4.0 % (0.5 g, at a pressure not exceeding 0.67 kPa, 100 °C, 6 hours).

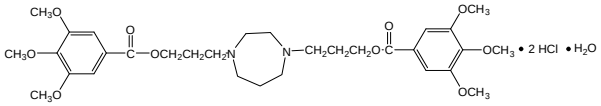
Assay Weigh accurately about 0.2 g of Dihydroergotamine Mesilate, dissolve in 170 mL of a mixture of acetic anhydride and acetic acid (100) (10 : 1) and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 13.596 mg of $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dilazep Hydrochloride Hydrate



$C_{31}H_{44}N_2O_{10} \cdot 2HCl \cdot H_2O$: 695.63

3-[4-[3-(3,4,5-Trimethoxybenzoyl)oxypropyl]-1,4-diazepan-1-yl]propyl3,4,5-trimethoxy-benzoate hydrate dihydrochloride [*20153-98-4,* anhydride]

Dilazep Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of dilazep hydrochloride ($C_{31}H_{44}N_2O_{10} \cdot 2HCl$), calculated on the dried basis.

Description Dilazep Hydrochloride Hydrate is a white, crystalline powder and is odorless.

Dilazep Hydrochloride Hydrate is freely soluble in acetic acid (100) or in chloroform, soluble in water, slightly soluble in ethanol (95) or in acetic anhydride and practically insoluble in ether.

Melting point—200 ~ 204 °C. Immerse the sample in a bath of 110 °C and raise the temperature at the rate of about 3 °C per minute from 140 °C to 150 °C, about 10 °C per minute from 160 °C to 195 °C and about 1 °C per minute from 195 °C.

Identification (1) Take 1 mL of a solution of Dilazep Hydrochloride Hydrate (1 in 100), add 0.1 mL of a solution of hydroxylamine hydrochloride (1 in 10) and 0.1 mL of 8 mol/L potassium hydroxide TS and warm in a water-bath of 70 °C for 10 minutes**.** After cooling, add 0.5 mL of dilute hydrochloric acid and 0.1 mL of iron (III) chloride TS: a purple color is observed.

(2) Take 5 mL of a solution of Dilazep Hydrochloride Hydrate (3 in 500), add 0.3 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Determine the absorption spectra of solutions of Dilazep Hydrochloride Hydrate and Dilazep Hydrochloride Hydrate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Dilazep Hydrochloride Hydrate and Dilazep Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(5) A solution of Dilazep Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 20 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.5 g of Dilazep Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Dilazep Hydrochloride Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Dilazep Hydrochloride Hydrate according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.40 g of Dilazep Hydrochloride Hydrate in 10 mL of chloroform and use this solution as the test solution**.** Pipet 1.0 mL of the test solution, add chloroform to make exactly 200 mL and use this solution as the standard solution**.** Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography.

Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, dichloromethane and hydrochloric acid (500 : 200 : 100 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying 2.0 ~ 3.0 % (1 g, 105 °C, 3 hours).

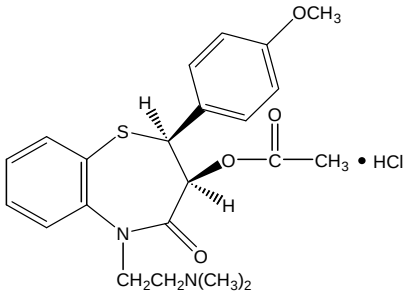
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Dilazep Hydrochloride Hydrate dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.881 mg of $C_{31}H_{44}N_2O_{10}\cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Diltiazem Hydrochloride



$C_{22}H_{26}N_2O_4S\cdot HCl$: 450.98

[(2*R*,3*R*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl]ethanoate hydrochloride [*33286-22-5*]

Diltiazem Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S\cdot HCl$).

Description Diltiazem Hydrochloride appears as white crystals or crystalline powder and is odorless.
Diltiazem Hydrochloride is very soluble in formic acid, freely soluble in water, in methanol or in chloroform, sparingly soluble in acetonitrile, slightly soluble in ethanol (99.5) or acetic anhydride and practically insoluble in ether.

Identification (1) Dissolve 50 mg of Diltiazem Hydrochloride in 1 mL of 1 mol/L of hydrochloric TS, add 2 mL of ammonium thiocyanate-cobalt nitrate TS and 5 mL of chloroform, shake well and allow to stand slowly: a blue color is observed in the chloroform layer.

(2) Proceed as directed under the Oxygen Flask Combustion Method with 30 mg of Diltiazem Hydrochloride, using 20 mL of water as the absorbing liquid and prepare the test solution: the test solution responds to the Qualitative Tests (1) for sulfate.

(3) Dissolve 10 mg each of Diltiazem Hydrochloride and Diltiazem Hydrochloride RS in 0.01 mol/L hydrochloric acid TS to make 100 mL each. Pipet 2 mL each of these solutions and add 0.01 mol/L hydrochloric acid TS to make 20 mL each. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Diltiazem Hydrochloride and Diltiazem Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(5) A solution of Diltiazem Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: +115 ~ +120° (after drying, 0.20 g , water, 20 mL, 100 mm).

Melting Point 210 ~ 215 °C (with decomposition).

pH Dissolve 1.0 g of Diltiazem Hydrochloride in 100 mL of water: the pH of this solution is between 4.3 and 5.3.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Diltiazem Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 1.0 g of Diltiazem Hydrochloride. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Diltiazem Hydrochloride according to Method 2 and perform the test**.** Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Place 1.0 g of Ditiazem Hydrochloride in a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the neck of the flask and heat cautiously until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat and repeat this procedure twice. Add several 2 mL volumes of strong hydrogen peroxide water and heat until the solution changes colorless to pale yellow. After cooling, add 2 mL of saturated solution of ammonium oxalate and heat again until white fumes are evolved.

After cooling, add water to make 5 mL, use this solution as the test solution and perform the test: the test solution has no more color than the following control solution (not more than 2 ppm).

Control solution—Proceed in the same manner as the test solution without Diltiazem Hydrochloride, add 2.0 mL of standard arsenic solution and water to make 5 mL and proceed in the same manner as the test solution.

(5) ***Related substances***—Dissolve 50 mg of Diltiazem Hydrochloride in 50 mL of diluted ethanol (99.5) (4 in 5) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted ethanol (99.5) (4 in 5) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by automatic integration method: the total peak area of peaks other than the peak of diltiazem obtained from the test solution is not more than 3/5 of the peak area of diltiazem obtained from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column Temperature: A constant temperature of about 50 °C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of d-camphorsulfonic acid in 500 mL of water and filter using a membrane filter (0.4 μm in pore size). Add 250 mL each of acetonitrile and methanol to the filtrate and adjust the solution to a pH of 6.6 by adding sodium acetate trihydrate. Make any necessary adjustment.

Flow rate: Adjust the flow rate so that the retention time of diltiazem is about 9 minutes.

System suitability

Test for required detection: To exactly 2 mL of the standard solution, add diluted ethanol (99.5) (4 in 5) to make exactly 10 mL. Confirm that the peak area of diltiazem obtained from 20 μL of this solution is equivalent to 15 to 25 % of that of diltiazem obtained from 20 μL of the standard solution.

System performance: Weigh 30 mg of Diltiazem Hydrochloride, 20 mg of d-3-hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepin-4-(5H)-one hydrochloride (i.e., deacetylated form) and 20 mg of phenylbenzoate, dissolve in 160 mL of ethanol (99.5) and add water to make 200 mL. Using 20 μL of this solution, perform the test as directed under Liquid Chromatography according to the above operating conditions: the deacetylated form, diltiazem and phenylbenzoate are eluted in this order and the resolution of the deacetylated form and diltiazem and the resolution of diltiazem and phenyl benzoate are not less than 2.5, respectively.

System repeatability: When the test is repeated 6 times each with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0 %.

Time span of measurement: About twice as long as the retention time of diltiazem after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.7 g of Diltiazem Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 45.10 mg of $C_{22}H_{26}N_2O_4S \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dilute Hydrochloric Acid

Dilute Hydrochloric Acid contains not less than 9.5 w/v % and not more than 10.5 w/v % of hydrogen chloride (HCl: 36.46).

Description Dilute Hydrochloric Acid is a colorless liquid. Dilute Hydrochloric Acid is odorless and has a strong acid taste.

Specific gravity d_{20}^{20}—About 1.05.

Identification A solution of Dilute Hydrochloric Acid (1 in 30) changes blue litmus paper to red and responds to the Qualitative Tests for chloride.

Purity (1) ***Bromine or chlorine***—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS and shake well: the chloroform layer remains colorless.

(2) ***Bromine or chlorine***—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform and shake for 1 minute: the chloroform layer remains free from a purple color.

(3) ***Sulfite***—Take 3.0 mL of Dilute Hydrochloric

Acid, add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(4) ***Sulfate***—Take 3.0 mL of Dilute Hydrochloric Acid, add 5 mL of water and 5 drops of barium chloride TS and allow to stand for 1 hour: no turbidity is produced.

(5) ***Heavy metals***—Evaporate 9.5 mL of Dilute Hydrochloric Acid in a water-bath to dryness, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(6) ***Mercury***—Dilute 80 mL of Dilute Hydrochloric Acid with water to make exactly 100 mL and use this solution as the test solution. Perform the test with this solution according to the Atomic Absorption Spectrophotometry (cold vapor type). Place the test solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air and read the absorbance, A_T, of the test solution after the recorder reading has risen rapidly and become constant at a wavelength of 253.7 nm. Separately, to 8 mL of standard mercury solution, add water to make exactly 100 mL and read the absorbance, A_S, of the solution obtained by the same procedure as used for the test solution: A_T is smaller than A_S (not more than 0.01 ppm).

(7) ***Arsenic***— Prepare the test solution 4.0 mL of Dilute Hydrochloric Acid according to Method 1 and perform the test (not more than 0.5 ppm).

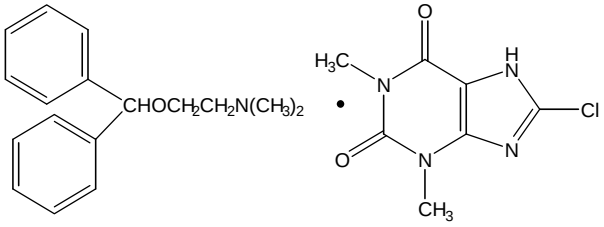
Residue on Ignition Pipet 10 mL of Dilute Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness and ignite: the weight of the residue is not more than 1.0 mg.

Assay Measure exactly 10 mL of Dilute Hydrochloric Acid and dilute with 20 mL of water. Titrate with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS
= 36.461 mg of HCl

Containers and Storage ***Containers***—Tight containers.

Dimenhydrinate



$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$: 469.96

2-Benzhydryloxy-*N,N*-dimethylethanamine;8-chloro-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione [*523-87-5*]

Dimenhydrinate, when dried, contains not less than 53.0 % and not more than 55.5 % of diphenhydramine ($C_{17}H_{21}NO$: 255.35) and not less than 44.0 % and not more than 47.0 % of 8-chlorotheophylline ($C_7H_7ClN_4O_2$: 214.61).

Description Dimenhydrinate is a white, crystalline powder, is odorless and has a bitter taste.
Dimenhydrinate is very soluble in chloroform, freely soluble in ethanol (95) and slightly soluble in water or in ether.

Identification (1) Dissolve 0.5 g of Dimenhydrinate in 30 mL of dilute ethanol, add 30 mL of water and use this solution as the test solution. Transfer 30 mL of the test solution to a separatory funnel and add 2 mL of ammonia solution (28). Extract with two 10 mL volumes of ether, combine the ether extracts, wash the combined extracts with 5 mL of water and then extract the combined extracts with 15 mL of diluted hydrochloric acid (1 in 100). With this water layer, perform the following tests.

(i) Take 5 mL of this acid extract, add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(ii) Take 10 mL of this acid extract, add 10 mL of 2,4,6-trinitrophenol TS and allow to stand for 30 minutes. Collect the precipitate by filtrating, recrystallize from dilute ethanol and dry at 105 °C for 30 minutes: the crystals melt between 128 °C and 133 °C.

(2) Take 30 mL of the test solution obtained in the Identification (1), add 2 mL of dilute sulfuric acid and cool for 30 minutes. Scratch the inside wall of the container frequently to facilitate crystallization: the white crystals are produced. Filter and wash the white crystals with a small amount of ice-cooled water. Dry the crystals for 1 hour at 105 °C: the crystals melt between 300 °C and 305 °C with decomposition.

(3) Take 10 mg of the crystals obtained in the Identification (2), add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid and evaporate on a water-bath to dryness: the residue shows a yellow-red color. When the dish containing the residue is held over a vessel containing 2 to 3 drops of ammonia TS, the color changes to red-purple, which is discharged on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Mix well 50 mg of crystals obtained in the Identification (2) with 0.5 g of sodium peroxide in a nickel crucible and heat until the mass melts. Cool, dissolve the melted mass in 20 mL of water and acidify with dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Melting Point 102 ~ 107 °C.

Purity (1) ***Chloride***—Transfer 50 mL of the filtrate obtained in the Assay (2) to a Nessler tube, add 1 mL of nitric acid and allow to stand for 5 minutes: the turbidity of the solution is not greater than that of the following control solution.

Control solution—Dilute 0.25 mL of 0.01 mol/L hydrochloric acid VS with 6 mL of dilute nitric acid and with water to make 50 mL, add 1 mL of silver nitrate TS and allow to stand for 5 minutes (not more than 0.044 %).

(2) ***Bromide and iodide***—Place 0.10 g of Dimenhydrinate in a glass-stoppered test tube and add 50 mg of sodium nitrite, 10 mL of chloroform and 10 mL of dilute hydrochloric acid. Stopper, shake well and allow to stand: the chloroform layer remains colorless.

Loss on Drying Not more than 0.5 % (3 g, in vacuum, P_2O_5, 24 hours).

Residue on Ignition Not more than 0.3 % (1 g).

Assay (1) ***Diphenhydramine***—Weigh accurately about 0.5 g of Dimenhydrinate, previously dried, transfer to a separatory funnel and add 50.0 mL of water, 3 mL of ammonia TS and 10 g of sodium chloride. Extract with six 15 mL volumes of ether with shaking, combine the ether extracts and wash the combined ether extracts with three 50 mL volumes of water. To the ether extracts, add 25.0 mL of 0.05 mol/L sulfuric acid and 25 mL of water. Shake thoroughly and evaporate the ether gently. Cool and titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS
= 25.536 mg of $C_{17}H_{21}NO$

(2) ***8-Chlorotheophylline***—Weigh accurately about 0.8 g of Dimenhydrinate, previously dried, transfer to a 200 mL volumetric flask, add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10) and heat in a water-bath for 5 minutes. Add 25.0 mL of 0.1 mol/L silver nitrate VS, heat in a water-bath for 15 minutes with occasional shaking, cool and add water to make exactly 200 mL. Allow to stand overnight to settle the precipitate and filter through a dry filter paper. Discard the first 20 mL of filtrate, measure exactly 100 mL of the subsequent filtrate, acidify with nitric acid, add 3 mL of nitric acid and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 21.461 mg of $C_7H_7ClN_4O_2$

Containers and Storage ***Containers***—Well-closed containers.

Dimenhydrinate Tablets

Dimenhydrinate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$: 469.96).

Method of Preparation Prepare as directed under Tablets, with Dimenhydrinate.

Identification (1) Weigh a portion of Dimenhydrinate Tablets, previously powdered, equivalent to 0.5 g of Dimenhydrinate according to the labeled amount, with 25 mL of warm ethanol, mix with grinding and filter. Dilute the filtrate with 40 mL of water and filter again. Use the filtrate as the test solution. Transfer 30 mL of the test solution to a separatory funnel and proceed as directed in the Identification (1) under Dimenhydrinate.

(2) With 30 mL of the test solution obtained in (1), proceed as directed in the Identification (2), (3) and (4) under Dimenhydrinate.

Dissolution Test Perform the test with 1 tablet of Dimenhydrinate Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 15 minutes after the start of the test and filter through a membrane filter with pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$), and use this solution as the test solution. Separately, weigh accurately about 28 mg of Dimenhydrinate RS (previously dried in vacuum over P_2O_5 for 24 hours) and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Dimenhydrinate Tablets in 15 minutes is not less than 85 %.

Dissolution rate (%) with respect to the labeled amount of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$)
= Amount (mg) of Dihydrinate RS

$$\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

C: Labeled amount (mg) of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

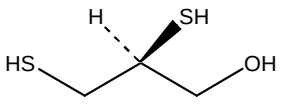
Assay Weigh accurately and powder not less than 20 Dimenhydrinate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$), transfer to a flask, add 80 mL of methanol, shake well to mix, add methanol again to make exactly 100 mL and filter. Pipet 5 mL of the filtrate, add 5.0 mL of the internal standard solution and use this solution as the test solution. Separately, weigh accurately about 25 mg of Dimenhydrinate RS, add methanol to make exactly 50 mL. Pipet 5 mL, add 5.0 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed in the Assay under Dimenhydrinate Injection.

$$\text{Amount (mg) of dimenhydrinate } (C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2) = \text{Amount (mg) of Dimenhydrinate RS} \times \frac{A_T}{A_S} \times 2$$

Internal standard solution—A solution of 2-hydroxy benzylalcohol in methanol (2.0 mg/mL).

Containers and Storage ***Containers***—Well-closed containers.

Dimercaprol



and enantiomer

$C_3H_8OS_2$: 124.23

2,3-Disulfanylpropan-1-ol [*59-52-9*]

Dimercaprol contains not less than 98.5 % and not more than 101.5 % of dimercaprol ($C_3H_8OS_2$).

Description Dimercaprol is a colorless to pale yellow liquid, and has a mercaptan-like, disagreeable odor.
Dimercaprol is soluble in peanut oil and sparingly soluble in water.
Dimercaprol is miscible with methanol or with ethanol (99.5).
Dimercaprol shows no optical rotation.

Identification (1) Add 1 drop of Dimercaprol to a mixture of 1 drop of a solution of cobaltous chloride hexahydrate (1 in 200) and 5 mL of water: a yellow-brown color is observed.

(2) Determine the infrared spectra of Dimercaprol and Dimercaprol RS as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20} : 1.570 ~ 1.575.

Specific Gravity d_{20}^{20} : 1.238 ~ 1.248.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 mL of Dimercaprol in 20 mL of peanut oil: the solution is clear and colorless to pale yellow.

(2) ***Bromide***—Take 2.0 g of Dimercaprol, add 25 mL of dilute potassium hydroxide-ethanol TS and heat in a water-bath under a reflux condenser for 2 hours. Evaporate the ethanol with a current of warm air, add 20 mL of water and cool. Add a mixture of 10 mL of hydrogen peroxide (30) and 40 mL of water, boil gently under a reflux condenser for 10 minutes and filter rapidly after cooling. Wash the residue with two 10 mL volumes of water, combine the washings with the filtrate, add 10 mL of dilute nitric acid and 5.0 mL of 0.1 mol/L silver nitrate VS and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.
Not more than 1.0 mL of 0.1 mol/L silver nitrate VS is consumed.

(3) ***Heavy metals***—Proceed with 1.0 g of Dimercaprol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***1,2,3-Trimercaptopropane and other related substances***—Weigh accurately 250 mg of hydrogen sulfide-free Dimercaprol, add the mobile phase to make exactly 5 mL and use this solution as the test solution. Use lead acetate paper to test for the presence of hydrogen sulfide. If the paper darkens, this indicates the presence of hydrogen sulfide: Remove hydrogen sulfide with the aid of a current of dry, oxygen-free nitrogen or carbon dioxide until a fresh strip of paper does not darken. Weigh accurately 20 g of 100-mesh silicic acid for column chromatography, dissolve in 20 mL of a solution prepared by dissolving 100 mg of sodium sulfite heptahydrate in 100 mL of pH 6.0 phosphate buffer, add 100 mL of chloroform and use this as the filler. Transfer the filler to a column for liquid column chromatography, 13 mm in diameter and 600 mm in length, packing firmly. Wash the column free from chloroform with the mobile phase, taking care to prevent the formation of air spaces. Transfer 2.0 mL of the test solution to the top of the prepared column and wash with the mobile phase. Collect a 20 mL of eluate containing 1,2,3-trimercaptopropane and use this as test solution (1), and collect a 3 mL of eluate and use this as test solution (2) for separation test. To each of test solution (1) and test solution (2), add the same volume of ethanol (95). Confirm that test solution (2) is not decolorized by 1 drop of 0.1 mol/L iodine VS, and titrate test solution (1) with 0.1 mol/L iodine VS until a

yellow color is produced. Separately, perform a blank determination with 20 mL of the solution that has been passed through the column, and make any necessary correction: the amount of 1,2,3-trimercaptopropane is not more than 1.5 %.

Each mL of 0.1 mol/L iodine VS
= 4.676 mg of $C_3H_8S_3$

Mobile phase—A mixture of diisopropyl ether and acid-washed hexane (1:1)

Diisopropyl ether—Place 100 mL of diisopropyl ether in a distilling flask and distil, retaining only that portion distilling between 68 °C and 69 °C. Do not evaporate to the point of dryness as diisopropyl ether forms explosive peroxides. Use only freshly distilled material.

Acid-washed hexane—Transfer 100 mL of hexane and 10 mL of sulfuric acid to a separatory funnel, shake for not less than 12 hours and allow the layers to separate. Transfer the hexane layer to a distilling flask and distill slowly, retaining only that portion distilling between 35 °C and 50 °C. Use only freshly distilled material.

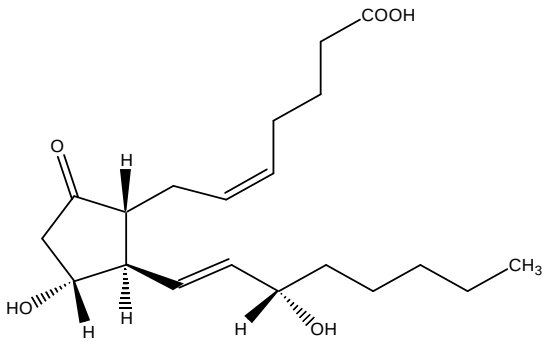
Assay Weigh accurately about 0.15 g of Dimercaprol into a glass-stoppered flask, dissolve in 10 mL of methanol and titrate immediately with 0.05 mol/L iodine VS until a pale yellow color is observed. Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 6.211 mg of $C_3H_8OS_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 5 ºC.

Dinoprostone



Prostaglandin E_2 $C_{20}H_{32}O_5$: 352.47

(*Z*)-7-((1*R*,2*R*,3*R*)-3-Hydroxy-2-((3*S*,*E*)-3-hydroxyoct-1-en-1-yl)-5-oxocyclopentyl)hept-5-enoic acid

[*363-24-6*]

Dinoprostone contains not less than 97.0 % and not more than 103.0 % of dinoprostone ($C_{20}H_{32}O_5$), calculated on the anhydrous basis.

Description Dinoprostone is a white or pale gray, crystalline powder and is odorless.

Identification (1) Determine the infrared spectra of Dinoprostone and Dinoprostone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: -82 ~ -90° (0.1 g, ethanol (95) 20 mL, 100 mm).

Purity ***Related substances***— Weigh 25.0 mg of Dinoprostone, add the mobile phase to make exactly 10 mL and use this solution as the test solution. Separately, weigh 25.0 mg of Dinoprostone RS, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (1). To 0.5 mL of the standard solution (1), add the mobile phase to make 50 mL and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solution (2) as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method and calculate the content of each related substance in the test solution: with respect to dinoprostone, the sum of the dinoprostone related substance I {15-oxo-1-dinoprostone} having a relative retention time of about 0.79, the dinoprostone related substance II {15-epi-dinoprostone} having a relative retention time of about 0.85, and the dinoprostone related substance III {8-isodinoprostone} having a relative retention time of about 0.90 is not more than 1.0 %, the dinoprostone related substance IV {5,6-trans-dinoprostone} having a relative retention time of about 1.15 is not more than 2.0 %, and the dinoprostone related substance V {(5*Z*,13*E*,15*S*)-15-hydroxy-9-oxoprosta-5,10,13-triene-1-oic acid} having a relative retention time of about 1.80 and the related substance VI {(5*Z*,13*E*,15*S*)-15-hydroxy-9-oxoprosta-5,8(12),13-triene-1-oic acid} having a relative retention time of about 1.90 are 1.0 % each. The content of any other related substance is 0.1 %. The peak areas of dinoprostone related substance I, dinoprostone related substance II, dinoprostone related substance V and dinoprostone related substance VI are determined by dividing the peak area obtained from the automatic integration method by response factors 5, 1.1, 5 and 1.43, respectively.

Content (%) of each related substance

$$= \frac{C}{W} \times \frac{1}{F} \times \frac{A_i}{A_S}$$

C: Concentration (μg/mL) of Dinoprostone RS in the standard solution (2)

W: Amount (mg) of Dinoprostone to prepared the test solution

F: Relative response factor

A_i: Peak area for each related substance obtained from the test solution

A_S: Peak area for dinoprostone obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of methanol and 0.2 % acetic acid (100) (58:42)

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates is not less than 6000. The resolution between dinoprostone peak and any other adjacent peak in the chromatogram obtained from the injection of the test solution is not less than 1.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution (1) under the above operating conditions, the relative standard deviation of the areas of the principal peak is not more than 2.0 %.

Water Not more than 0.5 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Weigh accurately about 25 mg of Dinoprostone, add the mobile phase to make exactly 10 mL and use this solution as the test solution. Separately, weigh 25 mg of Dinoprostone RS, add the mobile phase to make exactly 10 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions directed in the Related substances under the Purity and determine the area of dinoprostone peak in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of dinoprostone } (C_{20}H_{32}O_5) = \text{Amount (mg) of Dinoprostone RS} \times \frac{A_T}{A_S}$$

System suitability

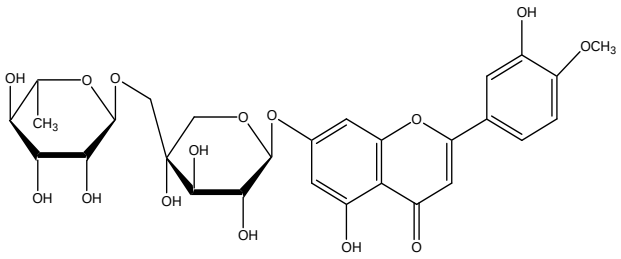
System performance: When the procedure is run with 20 μL of the standard solution according to the operating condition directed in related substances under Purity, the resolution between dinoprostone peak and any other adjacent peak is not less than 1.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution according to the operating condition directed in related substances under Purity, the relative standard deviation of the areas of the principal peak is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Diosmin



$C_{28}H_{32}O_{15}$: 608.55

5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyl-oxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one [*520-27-4*]

Diosmin contains not less than 90.0 % and not more than 102.0 % of diosmin ($C_{28}H_{32}O_{15}$), calculated on the anhydrous basis.

Description Diosmin is a pale grayish-yellow or light yellow powder.
Diosmin is soluble in dimethylsulfoxide and practically insoluble in water or in ethanol (95).
Diosmin is soluble in dilute sodium hydroxide TS.
Diosmin is hygroscopic.

Identification (1) Determine the infrared spectra of Diosmin and Diosmin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.
(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Diosmin according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of standard

lead solution (not more than 20 ppm).

(2) ***Iodine***—Prepare the test solution with 1.0 g of Diosmin as directed under the Oxygen Flask Combustion Method, using 50 mL of 0.02 w/v % hydrazine solution as an adsorbing liquid. Separately, weigh accurately 1.66 g of potassium iodide, dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL and use this solution as the control solution. Weigh 20 g of potassium nitrate and dissolve in 0.1 mol/L nitric acid to make 100 mL. Transfer 30 mL of the solution to a beaker, immerse an iodide ion selective electrode, stir for 10 minutes until the potential of the solution is stable (n_{T1}). Add 1 mL of the test solution and measure the potential (n_{T2}). Separately, weigh 20 g of potassium nitrate, dissolve in 0.1 mol/L nitric acid to make 100 mL. Transfer 30 mL of the solution to a beaker, immerse an iodide ion selective electrode, stir for 10 minutes until the potential of the solution is stable (n_{R1}). Add 80 μL of the control solution and measure the potential (n_{R2}). The absolute value, $|n_{T2}-n_{T1}|$, is less than the absolute value, $|n_{R2}-n_{R1}|$.

(3) ***Related substances***—Weigh accurately 25 mg of Diosmin, dissolve in dimethylsulfoxide to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately 25 mg of Diosmin RS, dissolve in dimethylsulfoxide to make exactly 25 mL. Pipet 5 mL of this solution, add dimethylsulfoxide to make 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks in these solutions by the automatic integration method. Multiply the peak areas by 0.38 and 0.61 for the related substances I and VI, respectively, for the calculation of the content of the related substances: the area of the related substance I peak obtained from the test solution is not more than 0.2 times the area of the principal peak obtained from the standard solution (1 %), the area of the related substance II peak from the test solution is not more than the area of the principal peak from the standard solution (5 %) and the individual area of the related substances III, V and VI peaks from the test solution is not more than 0.6 times the area of the principal peak from the standard solution. In addition, the area of the peak of any other related substance from the test solution is not more than 0.2 times the area of the principal peak from the standard solution (1 %), the area of the related substance I peak and total area of other related substances from the test solution are 0.2 times the area of the principal peak from the standard solution, and total area of the related substance peaks is not more than twice the area of the principal peak from the standard solution (10 %). Disregard any peak having the area not more than 0.02 times (0.1 %) the area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: 40 ºC.

Mobile phase: A mixture of water, methanol, acetic acid (100) and acetonitrile (66:28:6:2).

Flow rate: 1.5 mL/minute.

Relative retention time: The reference retention time for diosmin peak is about 4.6 min. The retention times for the related substances I, II, III, IV, V and VI are about 0.5, 0.6, 0.8, 2.2, 2.6 and 4.5 minutes, respectively.

System suitability

System performance: Weigh 25 mg of Diosmin and dissolve in dimethylsulfoxide to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the related substance II and the related substance III is not less than 2.5.

Water Not more than 6.0 % (0.3 g, volumetric titration, direct titration).

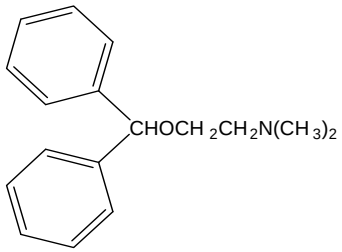
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately 25 mg each of Diosmin and Diosmin RS, dissolve in dimethylsulfoxide to make 25 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions in the Related substances under the Purity and determine the area of the principal peak of the test solution, A_T, and the standard solution, A_S.

$$\text{Amount (mg) of diosmin } (C_{28}H_{32}O_{15}) = \text{Amount (mg) of Diosmin RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Diphenhydramine



$C_{17}H_{21}NO$: 255.36

2-Benzhydryloxy-*N,N*-dimethylethanamine [*58-73-1*]

Diphenhydramine contains not less than 96.0 % and not more than 101.0 % of diphenhydramine ($C_{17}H_{21}NO$).

Description Diphenhydramine is a clear, pale yellow to yellow liquid, has a characteristic odor and has a burning taste at first, followed by slight sensation of numbness on the tongue.
Diphenhydramine is miscible with ethanol (95), with acetic acid (100), with acetic anhydride, or with ether.
Diphenhydramine is very slightly soluble in water.

Boiling point—About 162 ºC (in vacuum, 0.67 k Pa).

Refractive index n_D^{20} : About 1.55.

Diphenhydramine is gradually affected by light.

Identification (1) Take 50 mg of Diphenhydramine and add 2 mL of sulfuric acid: an orange-red precipitate is produced immediately and its color changes to red-brown on standing. Add carefully 2 mL of water to this solution: the intensity of the color changes, but the color tone does not change.

(2) Dissolve 0.1 g of Diphenhydramine in 10 mL of dilute ethanol, add an excess of a saturated solution of 2,4,6-trinitrophenol in dilute ethanol with stirring and cool in ice. Collect the produced crystals, recrystallize from dilute ethanol and dry at 105 ºC for 30 minutes: the crystals melt between 128 ºC and 133 ºC.

Specific Gravity d_{20}^{20} : 1.103 ~ 1.020.

Purity (1) ***-Dimethylaminoethanol***—Dissolve 1.0 g of Diphenhydramine in 20 mL of ether and extract with two 10 mL volumes of water with thorough shaking. Combine the water extracts and add 2 drops of phenolphthalein TS and 1.0 mL of 0.05 mol/L sulfuric acid VS: no red color is observed.

(2) ***Benzohydrol***—Transfer 1.0 g of Diphenhydramine to a separatory funnel, dissolve in 20 mL of ether and extract with two 25 mL volumes of diluted hydrochloric acid (1 in 15) with thorough shaking. Separate the ether layer, evaporate slowly in a water-bath and dry in a desiccator (in vacuum, silica gel) for 2 hours: the weight of the residue is not more than 20 mg.

(3) ***Heavy metals***—Proceed with 1.0 g of Diphenhydramine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Residue on Ignition Not more than 0.1 % (1 g).

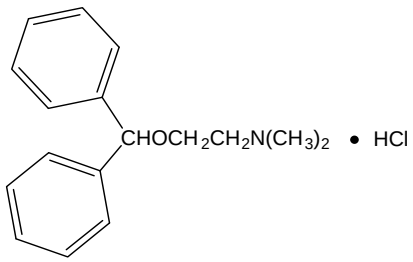
Assay Weigh accurately about 0.5 g of Diphenhydramine, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.536 mg of $C_{17}H_{21}NO$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and almost well-filled.

Diphenhydramine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$: 291.82

2-Benzhydryloxy-*N,N*-dimethyl-ethanamine hydrochloride [*147-24-0*]

Diphenhydramine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

Description Diphenhydramine Hydrochloride appears as white crystals or crystalline powder, is odorless and has a bitter taste, followed by sensation of numbness on the tongue.
Diphenhydramine Hydrochloride is very soluble in methanol or in acetic acid (100), freely soluble in water or in ethanol (95), sparingly soluble in acetic anhydride and practically insoluble in ether.
Diphenhydramine Hydrochloride is gradually affected by light.

Identification (1) Determine the absorption spectra of solutions of Diphenhydramine Hydrochloride and Diphenhydramine Hydrochloride RS in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Diphenhydramine Hydrochloride and Diphenhydramine Hydrochloride RS, both previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Diphenhydramine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Melting Point 166 ~ 170 ºC.

pH Dissolve 1.0 g of Diphenhydramine Hydrochlo-

ride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Diphenhydramine Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.20 g of Diphenhydramine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, methanol and ammonia solution (28) (10 : 4 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly iodine TS on the plate: the spots other than the principal spot from the test solution and the spot on the original point are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (2 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Diphenhydramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.182 mg of $C_{17}H_{21}NO{\cdot}HCl$.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Diphenhydramine Hydrochloride Capsules

Diphenhydramine Hydrochloride Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO{\cdot}HCl$: 291.82).

Method of Preparation Prepare as directed under Capsules, with Diphenhydramine.

Identification (1) Weigh a portion of Diphenhydramine Hydrochloride Capsules, equivalent to about 50 mg of Diphenhydramine Hydrochloride and perform the test as directed in the Identification (2) under Diphenhydramine Hydrochloride Injection.

(2) The retention of the principal peak in the chromatogram corresponds to that of the standard preparation, as obtained in the Assay.

Dissolution Test Take 1 capsule of Diphenhydramine Hydrochloride Capsules and perform the test using 500 mL of water as dissolution solution, at 100 revolutions per minute according to Method 1 under the Dissolution Test. After 30 minutes from the start of the test, take the dissolved solution and filter. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately a portion of Diphenhydramine Hydrochloride RS, previously dried at 105 ºC for 3 hours and dissolve in water at the same concentration as the test solution and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution, as directed according to Assay under Diphenhydramine Hydrochloride Injection.
The dissolution rate of Diphenhydramine Hydrochloride Capsules in 30 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately not less than 20 Diphenhydramine Hydrochloride Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of diphenhydramine hydrochloride ($C_{17}H_{21}NO{\cdot}HCl$), dissolve in water to make exactly 100 mL and filter**.** Perform the test as directed according to Assay under Diphenhydramine Hydrochloride Injection.

$$\text{Amount (mg) of diphenhydramine hydrochloride } (C_{17}H_{21}NO{\cdot}HCl) = \text{Amount (mg) of Diphenhydramine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Diphenhydramine Hydrochloride Injection

Diphenhydramine Hydrochloride Injection is a sterile solution of Diphenhydramine Hydrochloride in water for Injection. Diphenhydramine Hydrochloride Injection contains not less than 90.0 % and not more than 110.0 % at the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO{\cdot}HCl$: 291.82).

Method of Preparation Prepare as directed under Injections, with Diphenhydramine.

Description Diphenhydramine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) The retention of the principal peak in the chromatogram corresponds to that of the standard preparation.

(2) Take a volume of Diphenhydramine Hydrochloride Injection, equivalent to about 50 mg of Diphenhydramine Hydrochloride, add 0.03 mol/L sulfuric acid TS to make 25 mL, filter if necessary and use this solution as the test solution. Separately, to 50 mg of Diphenhydramine Hydrochloride RS, add 0.01 mol/L hydrochloric acid TS to make 25 mL and use this solution as the standard solution. To each of the test solution and the standard solution, add 2 mL of 1 mol/L sodium hydroxide TS and 4 mL of carbon disulfide and shake for 2 minutes. Filter by centrifugation, if necessary and proceed as directed in the solution method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 4.0 ~ 6.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 3.4 EU/mg of Diphenhydramine Hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Transfer an accurately pipeted portion of Diphenhydramine Hydrochloride Injection, equivalent to about 50 mg of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), add water to make exactly 100 mL and use this solution as the test solution. Weigh accurately about 50 mg of Diphenhydramine Hydrochloride, previously dried at 105 ºC for 3 hours, dissolve in water to make 100 mL and use this solution as the standard solution. Perform the test with 10 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine A_T and A_S, the peak area of diphenhydramine hydrochloride.

$$\text{Amount (mg) of diphenhydramine hydrochloride } (C_{17}H_{21}NO \cdot HCl) = \text{Amount (mg) of Diphenhydramine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Mobile Phase: Adjust pH to 6.5 by adding acetic acid (100) to a mixture of water, acetonitrile and triethylamine (50 : 50 : 0.5). make adjustment, if necessary

Flow rate: 1 mL/minute.

System suitability

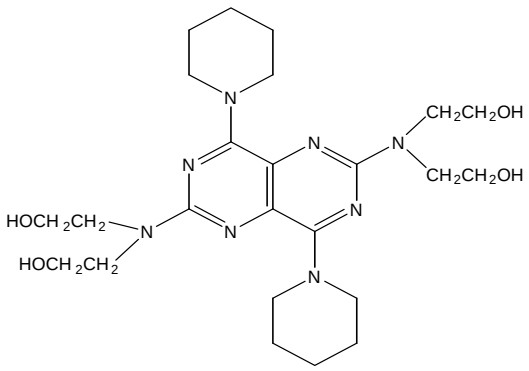
System performance: Dissolve 5 mg of benzophenone in 5 mL of acetonitrile and add water to make 500 mL. To 1 mL of this solution, add 5 mg of Diphenhydramine Hydrochloride and water to make 10 mL. When the procedure is run with 10 µL of this solution as directed under the above operating conditions, benzophenone and diphenhydramine are eluted in this order with the resolution between their peaks being greater than 2.0.

System repeatability: When the test is repeated 6 times each with 10 µL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Dipyridamole



$C_{24}H_{40}N_8O_4$: 504.63

2-[[2-[*bis*(2-Hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-6-yl]-(2-hydroxyethyl)amino]ethanol [*58-32-2*]

Dipyridamole, when dried, contains not less than 98.5 % and not more than 101.0 % of dipyridamole($C_{24}H_{40}N_8O_4$).

Description Dipyridamole appears as yellow crystals or crystalline powder, is odorless and has a slightly

bitter taste.
Dipyridamole is freely soluble in chloroform, sparingly soluble in methanol or in ethanol (99.5) and practically insoluble in water or in ether.

Identification (1) Dissolve 5 mg of Dipyridamole in 2 mL of sulfuric acid, add 2 drops of nitric acid and shake: a deep purple color is observed.

(2) Determine the absorption spectra of solutions of Dipyridamole and Dipyridamole RS in a mixture of methanol and hydrochloric acid (99 : 1) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Dipyridamole and Dipyridamole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 165 ~ 169 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Dipyridamole in 10 mL of chloroform: the solution is clear and shows a yellow color.

(2) ***Chloride***—Dissolve 0.5 g of Dipyridamole in 5 mL of ethanol (95) and 2 mL of 2 mol/L nitric acid and add 1 mL of silver nitrate TS: no turbidity or precipitate is produced.

(3) ***Heavy metals***—Proceed with 2.0 g of Dipyridamole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Dipyridamole according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 50 mg of Dipyridamole in 50 mL of the mobile phase and use this solution as the test solution. Pipet 0.50 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peak other than the peak of dipyridamole from the test solution is not larger than the peak area of dipyridamole from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 0.2 g of monobasic potassium phosphate in 200 mL of water and add 800 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of Dipyridamole is about 4 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dipyridamole obtained from 20 μL of the standard solution is between 2 mm and 6 mm.

System performance: Dissolve 7 mg of Dipyridamole and 3 mg of terphenyl in 50 mL of methanol. When the procedure is run with 20 μL of this solution as directed under the above operating conditions, dipyridamole and terphenyl are eluted in this order with the resolution between their peaks being not less than 5.0.

Time span of measurement: About 5 times as long as the retention time of dipyridamole.

Loss on Drying Not more than 0.2 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

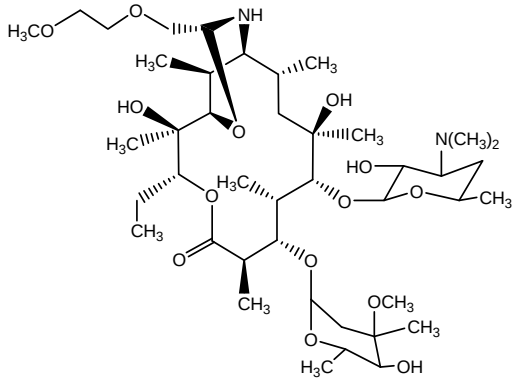
Assay Weigh accurately about 0.6 g of Dipyridamole, previously dried, dissolve in 70 mL of methanol and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.46 mg of $C_{24}H_{40}N_8O_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Dirithromycin



$C_{42}H_{78}N_2O_{14}$: 835.09

(1*S*,2*R*,4*R*,5*R*,6*S*,7*S*,8*R*,11*R*,12*R*,15*R*,17*S*)-5-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-11-ethyl-4,12-dihydroxy-7-

[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-15-(2-methoxyethoxymethyl)-2,4,6,8,12,17-hexamethyl-10,14-dioxa-16-azabicyclo[11.3.1]heptadecan-9-one [*62013-04-1*]

Dirithromycin contains not less than 96.0 % and not more than 102.0 % per mg of dirithromycin and epidirithromycin ($C_{42}H_{78}N_2O_{14}$), calculated on the anhydrous basis.

Description Dirithromycin is white to pale gray crystalline powder.
Dirithromycin is freely soluble in chloroform, soluble in methanol, slightly soluble in propanol or in acetonitrile, and very slightly soluble in water or in cyclohexane.

Identification (1) Determine the infrared spectra of Dirithromycin and Dirithromycin RS, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Determine the retention times according to the procedure of Assay: the retention time of the principal peak in the chromatogram obtained with test solution is the same as that of the principal peak in the chromatogram obtained with the standard solution

Crystallinity Test It meets the requirement.

Purity (1) ***Heavy metals***—Proceed with 0.1 g of Dirithromycin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related compounds***—Weigh accurately 0.1 g of Dirithromycin, dissolve in a mixture of acetonitrle and methanol (70:30), make to exactly 10 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Dirithromycin RS, dissolve in a mixture of acetonitrile and methanol (70:30) to make a solution containing 0.2 mg per mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method and calculate the content of each related substance according to the following equation: the content of 9-(*S*)-erythromycil
amine is not more than 1.5 %, the content of any other related substance is not more than 1.0 %, and the total amount of related substances is not more than 4.0 %. Dirithromycin 16*S*-epimer is not regarded as a related substance.

$$\text{Content (\%) of 9-(}S\text{)-erythromycilamine or other related substances} = \frac{C}{W} \times \frac{A_i}{A_S} \times 1000$$

C: Concentration (mg/mL) of dirithromycin in the standard solution
W: Amount (mg) of Dirithromycin taken
A_i: Peak area of each related substance obtained from the test solution
A_S: Peak area of dirithromycin (16*R*-epimer) from the standard solution

Operating conditions
Detector, column and mobile phase: Proceed as directed in the Assay.
Time span of measurement: About 3 times as long as the retention time of dirithromycin (16*R*-epimer)

(3) ***Dirithromycin 16s-epimer***—From the chromatogram obtained in the related substances test, determine the content (%) of dirithromycin 16*S*-epimer in dirithromycin according to the following formula (not more than 1.5 %).

$$\text{Content (\%) of dirithromycin 16}S\text{-epimer} = \frac{C}{W} \times \frac{A_E}{A_S SS} \times 1000 S$$

A_E: Peak area of dirithromycin 16*S*-epimer in the test solution
C: Concentration (mg/mL) of dirithromycin in the standard solution
W: Amount (mg) of Dirithromycin taken
A_S: Peak area of dirithromycin (16*R*-epimer) in the standard solution

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately about 20 mg each of Dirithromycin and Dirithromycin RS, dissolve each in a mixture of acetonitrile and methanol (70 : 30) to make exactly 10 mL and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of dirithromycin (16*R*-epimer).

$$\text{Amount (\%) of dirithromycin } (C_{42}H_{78}N_2O_{14}) = 1000 \times \frac{C}{W} \times \frac{A_T}{A_S} + P_E$$

C: Concentration [mg (potency)/mL] of dirithromycin in the standard solution
W: Amount (mg) Dirithromycin taken
P_E: Amount (%) of dirithromycin 16*S*-epimer calculated in the Purity (3)

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 205 nm)
Column: A stainless steel column, about 4.6 mm in

internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of acetonitirile, phosphate buffer solution (pH 7.5) and ethanol (44:37:19)

Flow rate: 2.0 mL per minutes.

System suitability

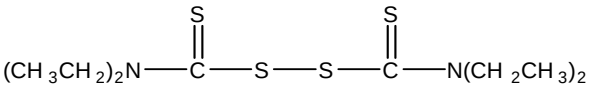
System performance: When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the relative retention times of 9-(*S*)-erythromycilamine, dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer are 0.7, 1.0 and 1.12, respectively. The resolution between the peaks of dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer is not less than 2.0, that between the peaks of dirithromycin (16*R*-epimer) and 9-(*S*)-erythromycilamine is not less than 5.0, and the symmetry factor of the dirithromycin (16*R*-epimer) peak is not more than 2.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the dirithromycin (16*R*-epimere) peak is not more than 1.0 %.

System suitability solution—Weigh accurately a suitable amount of Dirithromycin RS and dissolve in the mobile phase to make a solution containing 2.5 mg per mL. Keep this solution at room temperature for 24 hours. The solution is an equilibrium mixture of dirithromycin (16*R*-epimer), dirithromycin 16*S*-epimer and 9-(*S*)-erythromycilamine, and may be used for 1 month at room temperature.

Containers and Storage ***Containers***—Well-closed containers.

Disulfiram



$C_{10}H_{20}N_2S_4$: 296.54

Diethylcarbamothioylsulfanyl-*N,N*-diethyl-carbamo dithioate [*97-77-8*]

Disulfiram, when dried, contains not less than 99.0 % and not more than 101.0 % of disulfiram ($C_{10}H_{20}N_2S_4$).

Description Disulfiram appears as white to yellow crystals or crystalline powder.
Disulfiram is freely soluble in acetone or in toluene, slightly soluble in methanol or in ethanol (95) and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Disulfiram and Disulfiram RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Disulfiram and Disulfiram RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 70 °C ~ 73 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Disulfiram according to Method 2 and perform the test**.** Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Disulfiram according to method 4 and perform the test (not more than 2 ppm).

(3) ***Diethyldithiocarbamic acid***—Dissolve 0.10 g of Disulfiram in 10 mL of toluene and shake with 10 mL of diluted sodium carboxylate TS (1 in 20). Take the water layer separately, wash that with 10 mL of toluene, shake with 5 drops of a solution of cupuric sulfate (1 in 250) and 2 mL of toluene and allow to stand: a pale yellow color is not observed in the toluene layer.

(4) ***Related substances***—Dissolve 50 mg of Disulfiram in 40 mL of methanol, add water to make 50mL, and use this solution as the test solution . Pipet 1mL of the test solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of disulfiram from the test solution is not larger than the peak area of disulfiram from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 5 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of disulfiram is about 8 minutes.

Selection of column: Dissolve 50 mg of Disulfiram and 50mg of benzophenone in 40 mL of methanol, and add water to make 50 mL. To 1 mL of this solution add the mobile phase to make 200 mL. Proceed with 10 μL

of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of benzophenone and disulfiram in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of disulfiram obtained from 10 μL of the standard solution is 15 to 30 mm.

Time span of measurement: About 3.5 times of the retention time of disulfiram.

Loss on Drying Not more than 0.2 % (2 g, silica gel, 24 hours).

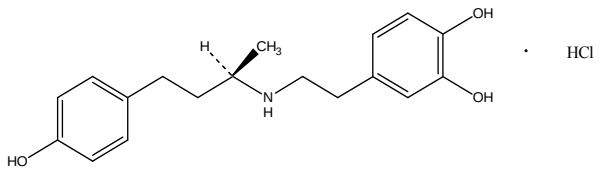
Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 0.2 g of Disulfiram, previously dried, in an iodine bottle, dissolve in 20 mL of acetone, add 1.5 mL of water and 1.0 g of potassium iodide and dissolve by shaking thoroughly. To this solution, add 3.0 mL of hydrochloric acid, stopper the bottle tightly, shake and allow to stand in a dark place for 3 minutes. Add 70 mL of water and titrate with 0.1 mol/L sodium thiosulfate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 14.83 mg of $C_{10}H_{20}N_2S_4$

Containers and Storage ***Containers***—Tight containers.

Dobutamine Hydrochloride



and enantiomer

$C_{18}H_{23}NO_3.HCl$: 337.84

(*RS*)-4-[2-[4-(4-Hydroxyphenyl)butan-2-ylamino]ethyl]benzene-1,2-diol hydrochloride [*49745-95-1*]

Dobutamine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of dobutaime hydrochloride ($C_{18}H_{23}NO_3.HCl$).

Description Dobutamine Hydrochloride is a white to very pale orange crystalline powder or grain.
Dobutamine Hydrochloride is freely soluble in methanol, sparingly soluble in water or in ethanol (95), and practically insoluble in ether.
A solution of Dobutamine Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the infrared spectra of Dobutamine Hydrochloride and Dobutamine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Dobutamine Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Melting Point 188 ~ 191 °C.

pH Dissolve 1.0 g of Dobutamine Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity **(1)** ***Clarity and color of solution***—Dissolve 0.5 g of Dobutamine Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Dissolve 1.0 g of Dobutamine Hydrochloride in 40 mL of water by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution add water to make 50 mL (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Dobutamine Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and formic acid (78:22:5) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g each of Dobutamine Hydrochloride and Dobutamine Hydrochloride RS, previously dried, dissolve in exactly 10 mL each of the internal standard solution, add diluted methanol (1 in 2) to make 50 mL each, and use these solutions as the test solution and the standard solution respectively. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dobutamine to that of the internal standard for the test solution and standard solution, respectively.

Amount (mg) of dobutamine hydrochloride ($C_{18}H_{23}NO_3$.HCl)

$$= \text{Amount (mg) of Dobutamine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of salicylamide in diluted methanol (1 in 2) (1 in 125).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.0 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of tartaric acid buffer solution, pH 3.0 and methanol (7:3).

Flow rate: Adjust the flow rate so that the retention time of dobutamine is about 7 minutes.

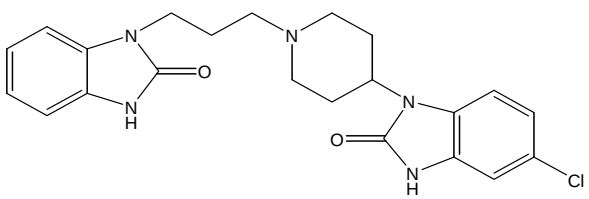
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, dobutamine and internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dobutamine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Domperidone



$C_{22}H_{24}Cl\ N_5O_2$: 425.91

5-Chloro-1-(1-(3-(2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-1-yl)propyl)piperidin-4-yl)-1*H*-benzo[d]imidazol-2(3*H*)-one [*57808-66-9*]

Domperidone, when dried, contains not less than 99.0 % and not than 101.0 % of domperidone ($C_{22}H_{24}Cl\ N_5O_2$).

Description Domperidone appears as white to pale yellow crystalline powder.

Domperidone is soluble in *N,N*-dimethylformamide, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 5 mg of Domperidone in 3 mL of methanol, add 0.1 mL each of 10 w/v % cobalt (II) nitrate hexahydrate solution and 10 w/v % calcium chloride dihydrate solution, mix and add 0.1 mL of 2 mol/L sodim hydroxide TS: a purple-blue color develops and precipitate is formed.

(2) Determine the infrared spectra of Domperidone and Domperidone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the absorption spectra of the solutions of Domperidone and Domperidone RS in a mixture of 2-propanol and 0.01 mol/L hydrochloric acid TS (9:1) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 244 ~ 248 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.2 g in 20 mL of *N,N*-dimethylformamide: the solution is colorless and clear.

(2) ***Heavy metals***—Proceed with about 2.0 g of Domperidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Prepare the solutions for the test immediately before use. Dissolve 0.1 g of Domperidone in 10 mL of *N,N*-dimethylformamide, and use this solution as the test solution. Pipet 1 mL of the test solution, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 5 mL of this solution, add *N,N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution (1). Perform the test with 10 μL each of *N,N*-dimethylformamide (as a blank solution), the test solution, and the standard solution (1) as directed under Liquid Chromatography according to the following conditions. Determine each peak area from each solution by the automatic integration method: the domperidone related substance I {5-chloro-1(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one} having a relative retention time of about 0.4, the domperidone related substance II {4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-formylpiperidine} having a relative retention time of about 0.65, the domeperidone related substance III {*cis*-4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidine-1-oxide} having a relative retention time of about 0.7, the domperidone related substance IV {5-chloro-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one} having a relative retention time of about 1.15, the domperidone related substance V {1-

[3-[4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1,3-dihydro-2*H*-benzimidazol-2-one} having a relative retention time of about 1.2, and the domperidone related substance VI {1,3-bis[3-[4-(5-chloro2-oxo-2,3-diihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-1,3-dihydro-2*H*-benzimidazol-2-one} having a relative retention time of about 1.3, with respect to domperidone, are each not larger than the peak area of domperidone from standard solution (1) (0.25 %). The peak area of other related substances is not larger than 0.4 times the peak area of domperidone from standard solution (1) (0.1 %). The sum of the peak areas of the related substances is not larger than 2 times the peak area of domperidone from standard solution (1) (0.5 %). Disregard any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak of domperidone from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and about 100 cm in length, packed with base-deactivated octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: At a flow rate of 1.5 mL per minute a mixture of ammonium acetate (5 g/L) and methanol (70 : 30), changing by linear gradient to methanol over 10 min, followed by elution with methanol for 2 min.

Flow rate: 1.5 mL/minute. Equilibrate the column for at least 30 min with methanol and then equilibrate at the initial mobile phase composition (a mixture of ammonium acetate (5 g/L) and methanol (70 : 30) for at least 5 minutes.

System suitability

Selection of column: Dissolve 10 mg of Domperidone RS and 15 mg of Droperidol RS in 100 mL of *N,N*-dimethylformamide and use this solution as the standard solution (2). Proceed with 10 μL of the standard solution (2) under the above operating conditions. Use a column from which the retention time for domperidone and droperidol is 6.5 minutes and 7 minutes, respectively, with the resolution between these peaks being not less than 2.0. If necessary, adjust the concentration of methanol in the mobile phase or adjust the concentration gradient.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of domperidone from 10 μL of the standard solution (1) is not less than 50.0 percent of the full scale of the recording chart.

Loss on Drying Not more than 0.5 % (1 g, 100 ~ 105 ºC, constant weight).

Residue on Ignition Not more than 0.1 % (1 g).

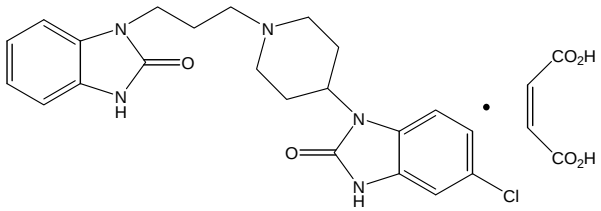
Assay Weigh accurately about 0.3 g of Domperidone, previously dried, dissolve in 50 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate with 0.1 mol/L perchloric acid (indictor: 0.2 mL of 1-naphthol-benzein TS) until the color changes from orange to green.

Each mL of 0.1 mol/L perchloric acid
= 42.59 mg of $C_{22}H_{24}Cl\ N_5O_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Domperidone Maleate



$C_{22}H_{24}Cl\ N_5O_2 \cdot C_4H_4O_4$: 541.98

(*Z*)-But-2-enedioic acid; 5-chloro-1-(1-(3-(2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-1-yl)propyl)piperidin-4-yl)-1*H*-benzo[d]imidazol-2(3*H*)-one [*83898-65-1*]

Domperidone Maleate contains not less than 99.0 % and not more than 101.0 % of domperidone maleate ($C_{22}H_{24}Cl\ N_5O_2 \cdot C_4H_4O_4$), calculated on the anhydrous basis.

Description Domperidone Maleate occurs as a white powder.

Domperidone Maleate is sparingly soluble in *N,N*-dimethylformamide, slightly soluble in methanol, and very slightly soluble in water or in ethanol (95).

Domperidone Maleate shows polymorphism.

Identification (1) Triturate 0.1 g of Domperidone Maleate with a mixture of 1 mL of strong sodium hydroxide solution and 3 mL of water. Shake with three quantities, each of 5 mL, of ether. Add 0.1 mL of the aqueous layer to the solution of 10 mg of resorcinol in 3 mL of sulfuric acid. Heat on a water-bath for 15 min. No color develops. To the remainder of aqueous layer add 2 mL of bromine solution. Heat on a water-bath for 15 min and then heat to boiling. Cool. Add 0.1 mL of the solution to a solution of 10 mg of resorcinol in 3 mL of sulfuric acid. Heat on a water-bath for 15 minutes. A red-purple color develops.

(2) Determine the infrared spectra of Domperidone Maleate and Domperidone Maleate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensity of absorption at the same wavenumbers. If the spectra obtained show differences, dissolve each

Domperidone Maleate and Domperidone Maleate RS in minimum volume of 2-propanol, evaporate to dryness on a water-bath and record new spectra using the residues.

(3) Dissolve 20 mg of Domperidone Maleate in 10 mL of methanol, and use this solution as the test solution. Separately, dissolve 20 mg of Domperidone Maleate RS in 10 mL of methanol, and use this as the standard solution (1). Dissolve 20 mg of Domperidone Maleate RS and 20 mg of Droperidole RS in 10 mL of methanol, and use this as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution, the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, 1,4-dioxane, acetic acid and ammonium acetate TS (40:40:20) to a distance of about 15 cm and air-dry the plate. Expose the plate with iodine vapor until spots appear and examine in day-light: the principal spot in the chromatogram obtained with the test solution corresponds in the size and the R_f value to that with the standard solution (1) and the two spots in the chromatogram obtained with the standard solution (2) are clearly separated with each other.

Purity (1) ***Clarity and color of solution***—Dissolve 0.2 g of Domperidone Maleate in 20 mL of *N,N*-dimethylformamide: the solution is clear and not darker than the mixture of 5 mL of the mixed solution of 1 w/v % hydrochloric acid, iron (III) chloride hexahydrate colorimetric stock solution and cobalt (II) chloride hexahydrate colorimetric stock solution (70:24:6) and 95 mL of 1 w/v % hydrochloric acid.

(2) ***Heavy metals***— Proceed with 1.0 g of Domperidone Maleate according to Method 4, and perform the test**.** Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***— Prepare the solution immediately before use. Dissolve 0.1 g of Domperidone Maleate in 10 mL of *N,N*-dimethylformamide, and use this solution as the test solution. Pipet 1 mL of the test solution, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 5 mL of this solution, add *N,N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution (1). Perform the test with 10 μL each of *N,N*-dimethylformamide (as a blank solution), the test solution, and the standard solution (1) as directed under Liquid Chromatography according to the following conditions. Determine each peak area from each solution by the automatic integration method: the area of peaks other than the peak of domperidone maleate from the test solution is not larger than the peak area of domperidone maleate from the standard solution (1) (0.25 %), and the total area of all peak other than the peak of domperidone maleate from the test solution is not larger than twice the peak area of domperidone maleate from the standard solution (1) (0.5 %). Disregard any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak of domperidone maleate from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and about 100 cm in length, packed with base-deactivated octadecylsilyl silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: At a flow rate of 1.5 mL per minute a mixture of ammonium acetate (5 in 1000) and methanol (70 : 30), changing by linear gradient to methanol over 10 min, followed by elution with methanol for 2 min.

Flow rate: 1.5 mL/minute. Equilibrate the column for at least 30 min with methanol and then equilibrate at the initial mobile phase composition (a mixture of ammonium acetate (5 in 1000) and methanol (70 : 30)) for at least 5 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of domperidone maleate from 10 μL of the standard solution (1) is not less than 50.0 percent of the full scale.

System performance: Dissolve 10 mg of Domperidone Maleate RS and 15 mg of Droperidol RS in 100 mL of *N,N*-dimethylformamide and use this solution as the standard solution (2). When the procedure is run with 10 μL of this solution, as directed under the above operating conditions, domperidone maleate and droperidol are eluted in this order with the resolution between their peaks being not less than 2.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, constant mass).

Residue on Ignition Not more than 0.1 % (1 g)

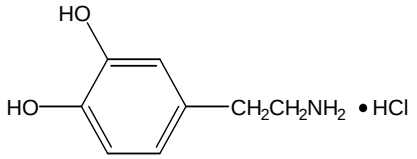
Assay Weigh accurately 0.4 g of Domperidone Maleate, previously dried, dissolve in 50 mL of a mixture of acetic acid (100). Titrate with 0.1 mol/L perchloric acid until the color changes from orange to green (indicator: 0.2 mL of 1-naphtholbenzein solution TS).

Each mL of 0.1 mol/L perchloric acid
= 54.20 mg of $C_{26}H_{28}ClN_5O_6$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Dopamine Hydrochloride



$C_8H_{11}NO_2 \cdot HCl$: 189.64

4-(2-Aminoethyl)benzene-1,2-diol hydrochloride
[*62-31-7*]

Dopamine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$).

Description Dopamine Hydrochloride appears as white crystals or crystalline powder.
Dopamine Hydrochloride is freely soluble in water or in formic acid, and sparingly soluble in ethanol (95).

Melting point—About 248 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Dopamine Hydrochloride and Dopamine Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (1 in 25000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Dopamine Hydrochloride and Dopamine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers

(3) A solution of Dopamine Hydrochloride (1 in 50) responds to the Qualitative Tests (1) for chloride.

pH Dissolve 1.0 g of Dopamine Hydrochloride in 50mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Dopamine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.8 g of Dopamine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Dopamine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***— Prepare the test solution with 1.0 g of Dopamine Hydrochloride according to Method 1 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Dopamine Hydrochloride in 10 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution, add water to make exactly 250 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 5 µL each of the test solution and the standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (16 : 8 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90 ºC for 10 minutes: the spots other than the principal spot form the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

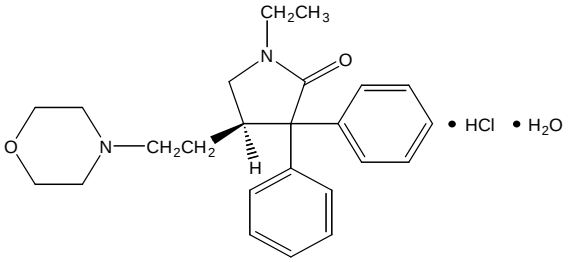
Assay Weigh accurately about 0.2 g of Dopamine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 15.0 mL of 0.1 mol/L perchloric acid VS and heat on a water-bath for 15 minutes. After cooling, add 50 mL of acetic acid (100) and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.964 mg of $C_8H_{11}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Doxapram Hydrochloride Hydrate



and enantiomer

$C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$: 432.98

(*RS*)-1-Ethyl-4-(2-morpholin-4-ylethyl)-3,3-diphenylpyrrolidin-2-one hydrate hydrochloride
[*7081-53-0*]

Doxapram Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of doxapram hydrochloride ($C_{24}H_{30}N_2O_2 \cdot HCl$: 414.97), calculated

on the anhydrous basis.

Description Doxapram Hydrochloride appears as white crystals or crystalline powder.
Doxapram Hydrochloride is freely soluble in methanol or in acetic acid (100), sparingly soluble in water, in ethanol (95), or in acetic anhydride and practically insoluble in ether.

Identification (1) Determine the absorption spectra of solutions of Doxapram Hydrochloride Hydrate and Doxapram Hydrochloride Hydrate RS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Doxapram Hydrochloride Hydrate and Doxapram Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Doxapram Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests for chloride.

Melting Point 218 ~ 222 ºC.

pH Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless

(2) ***Sulfate***—Perform the test with 1.0 g of Doxapram Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Doxapram Hydrochloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***— Prepare the test solution with 1.0 g of Doxapram Hydrochloride Hydrate according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.5 g of Doxapram Hydrochloride Hydrate in 10 mL of methanol and use this solution as the test solution. Pipet 3.0 mL of the test solution and add methanol to make exactly 100 mL. Pipet 5.0 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 6 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, formic acid, ethyl formate and methanol (8 : 3 : 3 : 2) to a distance of about 10 cm and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Water 3.5 ~ 4.5 % (0.5 g, volumetric titration, direct titration).

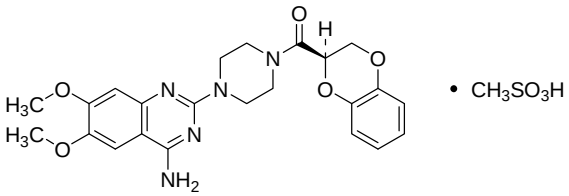
Residue on Ignition Not more than 0.3 % (1 g).

Assay Weigh accurately about 0.8 g of Doxapram Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.50 mg of $C_{24}H_{30}N_2O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Doxazosin Mesylate



and enantiomer

$C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$: 547.58

(*RS*)-[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(2,3-dihydro-1,4-benzo-dioxin-3-yl)methanone; methanesulfonic acid [*77883-43-3*]

Doxazosin Mesylate contains not less than 98.0 % and not more than 102.0 % of doxazosin mesylate ($C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$), calculated on the dried basis.

Description Doxazosin Mesylate appears as white to pale yellow crystalline powder.
Doxazosin Mesylate is freely soluble in dimethylsulfoxide, slightly soluble in water or in methanol and very slightly soluble in ethanol (99.5).
A solution of Doxazosin Mesylate in dimethylsulfoxide (1 in 20) shows no optical rotation.

Melting point—About 272 °C (with decomposition)

Identification (1) Determine the absorption spectrum of the solution of Doxazosin Mesylate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200000) as directed Ultraviolet-visible Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Doxazosin Mesylate as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Doxazosin Mesylate responds to the Qualitative Tests (2) for mesylate.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Doxazosin Mesylate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 20 mg of Doxazosin Mesylate in 5 mL of a mixture of methanol and acetic acid (100) (1:1) and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, water and acetic acid (100) (2:1:1) and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot at the R_f value of about 0.15 obtained from the test solution is not more intense than the spot from the standard solution, and no spots other than the principal spot and the spot at the R_f value of about 0.15 appear from the test solution.

Loss on Drying Not more than 1.0 % (1g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 25 mg each of Doxazosin Mesylate and Doxazosin Mesylate RS, previously dried, and dissolve separately in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of doxazosin in each solution.

$$\text{Amount (mg) of doxazosin mesylate } (C_{23}H_{25}N_5O_5 \cdot CH_4O_3S) = \text{Amount (mg) of Doxazosin Mesylate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 246 nm)

Column: A stainless steel column 3.9 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and acetonitrile (12:8:3)

Flow rate: Adjust the flow rate so that the retention time of doxazosin is about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of therotecial plates of the doxazosin peak is not less than 2000 with the symmetry factor being not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Doxazosin Mesylate Tablets

Doxazosin Mesylate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$: 451.48).

Method of Preparation Prepare as directed under Tablets, with Doxazosin Mesylate.

Identification Take a portion of powdered Doxazosin Mesylate Tablets, equivalent to about 5 mg of doxazosin according to the labeled amount, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake and centrifuge. Pipet 4 mL of the clear supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 244 nm and 248 nm.

Dissolution Test Perform the test with 1 tablet of Doxazosin Mesylate Tablets at 75 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer (pH 4.0) as the dissolution solution. After 15 minutes from the start of the test, take 20 mL or more of the dissolved solution and filter through a membrane with pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate and add the dissolution solution to make exactly V' mL so that each mL contains about 0.56 μg of doxazosin ($C_{23}H_{25}N_5O_5$) according to the labeled amount. Pipet 5 mL of this solution, add exactly 5 mL

of methanol and use this solution as the test solution. Separately, weigh accurately 21 mg of Dosazosin Mesylate RS, previously dried at 105 °C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of the dissolution solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of doxazosin in each solution. The dissolution rate of Doxazosin Mesylate Tablets in 15 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$)
= Amount (mg) of Doxazosin Mesylate RS

$$\times\frac{A_T}{A_S}\times\frac{V'}{V}\times\frac{1}{C}\times\frac{72}{25}\times 0.825$$

C: Labeled amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 246 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of methanol and buffer solution (55:45)

Buffer solution—Dissolve 3.4 g of potassium dihydrogen phosphate in 500 mL of water and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust the flow rate so that the retention time of doxazosin is about 5 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of doxazosin is not less than 1000 with the symmetry factor being not more than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

To 1 tablet of Doxazosin Mesylate Tablets, add 1 mL of water, shake, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL and shake for 30 minutes. Centrifuge, pipet V mL of the clear supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly V' mL so that each mL contains about 5 μg of doxazosin, and use this solution as the test solution. Separately, weigh accurately 30 mg of Doxazosin Mesylate RS, previously dried at 105 °C for 4 hours, and dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL and use this solution as the standard solution. Perform the test as directed in the Assay.

Amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$)
= Amount (mg) of Doxazosin Mesylate RS

$$\times\frac{A_T}{A_S}\times\frac{V'}{V}\times\frac{1}{50}\times 0.825$$

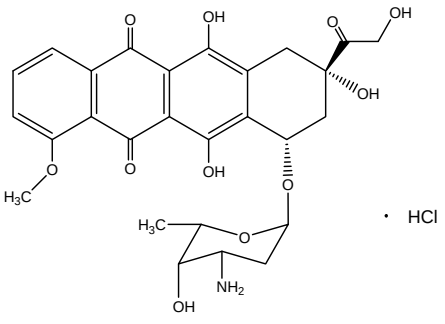
Assay Weigh accurately and powder not less than 20 Doxazosin Mesylate Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of doxazosin ($C_{23}H_{25}N_5O_5$), dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL and shake for 30 minutes. Centrifuge, pipet 4 mL of the clear supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately 24 mg of Doxazosin Mesylate RS, previously dried at 105 °C for 4 hours, and dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 246 nm as directed under Ultraviolet-visible Spectrophotometry, using 0.01 mol/L hydrochloric acid-methanol TS as the blank.

Amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$)
= Amount (mg) of Doxazosin Methylate RS

$$\times\frac{A_T}{A_S}\times\frac{1}{4}\times 0.825$$

Containers and Storage ***Containers***—Tight containers.

Doxorubicin Hydrochloride



$C_{27}H_{29}NO_{11}$·HCl: 579.98

(7*S*,9*S*)-7-[(2*R*,4*S*,5*S*,6*S*)-4-Amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dione hydrochloride [*25316-40-9*]

Doxorubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.
Doxorubicin Hydrochloride contains not less than 900 μg (potency) of per mg of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$·HCl), calculated on the anhydrous basis.

Description Doxorubicin Hydrochloride is orange crystalline powder.
Doxorubicin Hydrochloride is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Doxorubicin Hydrochloride (1 in 200) responds to the Qualitative Tests (1) for chloride.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +240 ~ +290° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 50 mg of Doxorubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Absorbance $E_{1\,cm}^{1\%}$ (495 nm): 200 ~ 230 (10 mg calculated on the anhydrous basis, methanol, 500 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 50 mg of Doxorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) ***Related substances***—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the test solution is not larger than 1/4 times the peak area of doxorubicin from the standard solution, and the total area of the peak other than doxorubicin is not larger than the peak area of doxorubicin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of doxorubicin obtained from 20 μL of this solution is equivalent to 3.5 % to 6.5 % of hat from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 with respect to doxorubicin, and doxorubicin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of doxorubicin.

Water Not more than 3.0 % (0.3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement when Doxorubicin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 2.50 EU/mg of doxorubicin hydrochloride when used in a sterile preparation.

Histamine It meets the requirement, when Doxorubicin Hydrochloride is used in a sterile preparation.

Weigh appropriate amount of Doxorubicin Hydrochloride, dissolve in water, make the solution so that each mL contains 2.0 mg (potency), use the solution as the test solution, and use 0.5 mL of the solution for the test.

Assay Weigh accurately an amount of Doxorubicin Hydrochloride, equivalent to about 2.0 mg (potency) according to the labeled potency, dilute with the mobile phase to render the concentration of 0.1 mg (potency) per mL and use this solution as the test solution. Separately, weigh accurately about 10 mg (potency) of Doxorubicin Hydrochloride RS, dilute with the mobile phase to render the concentration of 0.1 mg (potency) per mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of doxorubicin hydrochloride in the test solution and in the standard solution.

$$\text{Amount [μg (potency)] of doxorubicin hydrochloride } (C_{27}H_{29}NO_{11}\cdot HCl) = \text{Amount [μg (potency)] of Doxorubicin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 1 g of sodium lauryl sulfate in a mixture of water, acetontrile, ethanol (95) and phosphoric acid (540:290:170:2), and adjust to pH 3.6 ± 0.1 with 2 mol/L sodium hydroxide TS.

Flow rate: About 1.5 mL/minute

System suitability:

System performance: When the procedure is run with 20 μL of the resolution test solution under the above operating conditions, the relative retention time of doxorubicinone with respect to doxorubicin is about 0.6 with the resolution between these peaks being not less than 5.5, and the number of theoretical plates and the symmetry factor of the peak of doxorubicin are not less than 2250 and between 0.7 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 1.0 %.

Resolution test solution—Dissolve about 10 mg (potency) of Doxorubicin Hydrochloride in 5 mL of water, add 5 mL of phosphoric acid and allow to stand for about 30 minutes. Adjust the pH to 2.6 ± 0.1 with 2 mol/L sodium hydroxide TS (about 37 mL), add 15 mL of acetonitrile and 10 mL of methanol, mix, filter and use this solution as the resolution test solution. Keep some of this solution in a freezer and unfreeze and mix before use.

Containers and Storage ***Containers***—Tight containers.

Doxorubicin Hydrochloride for Injection

Doxorubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.
Doxorubicin Hydrochloride for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}\cdot HCl$: 579.98).

Method of Preparation Prepare as directed under Injections, with Doxorubicin Hydrochloride.

Description Doxorubicin Hydrochloride for Injection appears as orange powder or mass.

Identification Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 10 mg (potency) of doxorubicin hydrochloride according to the labeled amount, in methanol to make 100 mL. To 5 mL of this solution, add methanol to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 231 nm and 235 nm, between 250 nm and 254 nm, between 477 nm and 481 nm, between 493 nm and 497 nm, and exhibits a shoulder between 528 nm and 538 nm.

pH The pH of a solution, prepared by dissolving an amount of Doxorubicin Hydrochloride for Injection, equivalent to 50 mg (potency) of doxorubicin hydrochloride according to the labeled amount in 10 mL of water, is between 5.0 and 6.0.

Purity ***Clarity and color of solution***—Take exactly an amount of Doxorubicin Hydrochloride for Injection, equivalent to about 50 mg of doxorubicin hydrochloride according to the labeled amount, and dissolve in 10 mL of water: the solution is clear and red.

Water Not more than 4.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 2.50 EU/mg (potency) of doxorubicin hydrochloride.

Uniformity of Dosage Units It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Doxorubicin Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of doxorubicin hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use the solution as the test solution. Separately, weigh accurately an amount of Doxorubicin Hydrochloride RS, equivalent to 10 mg (potency), add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution.
Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography, and calculate ths ratios, Q_T and Q_S, of the peak area of doxorubicin to that of the internal standard.

Amount [μg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$·HCl)
= Amount [μg (potency)] of Doxorubicin Hydrochloride RS $\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000). To this solution add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, doxorubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5 and the symmetry factor of the peak of doxorubicin is between 0.8 and 1.2.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of doxorubicin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Doxorubicin Hydrochloride Injection

Doxorubicin Hydrochloride Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$·HCl : 579.98).

Method of Preparation Prepare as directed under Injections, with Doxorubicin Hydrochloride.

Description Doxorubicin Hydrochloride Injection appears as orange liquid.

Identification The retention time of the main peak obtained from the test solution in the Assay corresponds to the retention time of the main peak of the standard solution.

pH 2.5 ~ 3.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 2.2 EU/mg (potency) of doxorubicin hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Doxorubicin Hydrochloride Injection, equivalent to about 2.0 mg (potency) according to the labeled potency, dilute with the mobile phase to make a solution containing 0.1 mg (potency) per mL and use this solution as the test solution. Separately, weigh accurately about 10 mg (potency) of Doxorubicin Hydrochloride RS, dilute with the mobile phase to make a solution containing 0.1 mg (potency) per mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of doxorubicin in the test solution and the standard solution.

Amount [μg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$·HCl : 579.98)
= Amount [μg (potency)] of

$$\text{Doxorubicin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography.

Mobile phase: Dissolve 1 g of sodium lauryl sulfate in a mixture of water, acetontrile, ethanol (95) and phosphoric acid (540:290:170:2) and adjust the pH to 3.6 ± 0.1 with 2 mol/L sodium hydroxide TS.

Flow rate: About 1.5 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the resolution test solution under the above operating conditions, the relative retention time of doxorubicinone with respect to doxorubicin is about 0.6 with the resolution between these peaks being not less than 5.5. The number of theoretical plates of doxorubicin is 2250 with the symmetry factor being between 0.7 and 1.2.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, the relative standard deviation of the peak area of doxorubicin is not more than 1.0 %.

Resolution test solution—Dissolve about 10 mg (potency) of Doxorubicin Hydrochloride in 5 mL of water, add 5 mL of phosphoric acid and allow to stand for about 30 minutes. Adjust the pH to 2.6 ± 0.1 with 2 mol/L sodium hydroxide TS (about 37 mL), add 15 mL of acetonitrile and 10 mL of methanol, filter and use this solution as the resolution test solution. Keep some of this solution in a freezer and unfreeze and mix before use.

Containers and Storage ***Containers***—Hermetic containers.

Doxycycline Capsules

Doxycycline Capsules contain not less than 90.0 % and not more than 120.0 % of the labelend amount of doxycycline ($C_{22}H_{24}N_2O_8$: 444.44).

Method of Preparation Prepare as directed under Capsules, with Doxycycline Hydrate.

Identification (1) Weigh about 0.1 g (potency) each of Doxycycline Capsules and Doxycycline RS, add 100 mL of methanol, shake well, filter and use the clear supernatant liquid as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Heat a plate coated with octylsilanized silica gel for liquid chromatography, 0.25 mm in thickness, at 130 °C for 20 minutes and allow to cool. Spot the test solution and the standard solution on the plate while still warm, develop the plate with a mixture of 0.5 mol/L oxalic acid (previously adjusted to pH 2.0 with ammonium hydroxide), methanol and acetontrile (80:20:20) and air-dry the plate. Expose the plate to ammonia vapor for 5 minutes and examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution show the same R_f value as those obtained from the standard solution.

(2) The retention time of the principal peak obtained from the test solution under the Assay corresponds to that obtained from the standard solution.

Loss on Drying Not more than 5.0 % (0.1 g, 60 °C, 2 hours).

Dissolution Test Perform the test with 1 capsule of Doxycycline Capsules at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid TS as the dissolution solution. Take the dissolved solution after 60 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Doxycycline RS, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 268 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Doxycycline Capsules at 60 minutes is not less than 85 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount of doxycycline } (C_{22}H_{24}N_2O_8) = C_S \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of doxycycline ($C_{22}H_{24}N_2O_8$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test as directed in the Assay under Doxycycline Hyclate Hydrate. Weigh accurately the contents of not less than 20 Doxycycline Capsules. Weigh a portion of the combined contents, equivalent to about 100 mg (potency) according to the labeled potency of Doxycycline Capsules, add 20 mL of 0.1 mol/L hydrochloric acid, sonicate for 5 minutes, shake for 15 minutes to dissolve and add 0.01 mol/L hydrochloric acid to make exactly 100 mL. Filter through a membrane filter with pore size of not more than 0.5 μm

and use the filtrate as the test solution. Separately, weigh accurately about 10 mg (potency) of Doxycycline RS, add 6 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes to dissolve, make exactly 10 mL and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Doxycycline Hyclate Capsules

Doxycycline Hyclate Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$: 444.44).

Method of Preparation Prepare as directed under Capsules, with Doxycycline Hyclate Hydrate.

Identification (1) Weigh and powder the contents of 1 capsule of Doxycycline Hyclate Capsules and add 2 to 3 drops of sulfuric acid: a yellow color is observed.

(2) Weigh about 10 mg (potency) of powdered Doxycycline Hyclate Capsules, dissolve in 20 mL of water and add silver nitrate TS: the solution shows a white turbidity.

(3) The retention time of the main peak obtained from the test solution under the Assay is the same as that obtained from the standard solution.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 capsule of Doxycycline Hyclate Capsules at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution, with the distance between the bottom edge of the stirring blade and the inside bottom of the flask being maintained at 4.5 ± 0.5 cm during the test. Take the dissolved solution after 30 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Doxocycline RS, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Doxycycline Hyclate Tablets at 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of doxycycline ($C_{22}H_{24}N_2O_8$) in 1 capsule

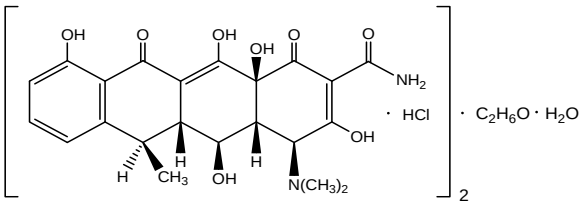
Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Doxycycline Hyclate Hydrate. Weigh accurately the contents of not less than 20 Doxycycline Hyclate Capsules. Weigh a portion of the combined contents, equivalent to about 100 mg (potency) according to the labeled potency of Doxycycline Hyclate Capsules, add 75 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes, shake for 15 minutes to dissolve and add 0.01 mol/L hydrochloric acid to make exactly 100 mL. Filter through a membrane filter with pore size of no more than 0.5 μm and use the filtrate as the test solution. Separately, weigh accurately about 10 mg (potency) of Doxycycline RS, add 6 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes to dissolve, make exactly 10 mL and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Doxycycline Hyclate Hydrate



$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$: 1025.89

(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide; ethanol; hydrate;dihydrochloride [*24390-14-5*]

Doxycycline Hyclate Hydrate is the hydrochloride of a derivative of oxytetracycline.

Doxycycline Hyclate Hydrate contains not less than 800 μg (potency) and not more than 920 μg (potency) per mg of doxycycline ($C_{22}H_{24}N_2O_8$: 444.43), calculated on the anhydrous basis and corrected by the amount of ethanol.

Description Doxycycline Hyclate Hydrate appears as yellow to dark yellow crystals or crystalline powder. Doxycycline Hyclate Hydrate is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification Determine the infrared spectra of Doxycycline Hyclate Hydrate and Doxycycline Hyclate Hydrate RS, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: -105 ~ -120° (0.25 g calculated on the anhydrous basis and corrected by the amount of alcohol, 0.01 mol/L hydrochloric acid-methanol TS, 25 mL, 100 mm). Determine within 5 minutes after the test solution is prepared.

pH The pH of a solution obtained by suspending 0.1 g of Doxycycline Hyclate Hydrate in 10 mL of water is between 2.0 and 3.0.

Absorbance $E_{1\,cm}^{1\%}$ (249 nm): 285 ~ 315 (10 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL).

Purity (1) ***Ethanol***—Weigh accurately about 1.0 g of Doxycycline Hyclate Hydrate, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately about 0.4 g of ethanol (99.5), add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard:the amount of ethanol is not less than 4.3 % and not more than 6.0 %.

$$\text{Amount (\%) of ethanol in Doxycycline Hyclate Hydrate} = \frac{\text{Amount (g) of ethanol}}{\text{Amount (g) of Doxycycline Hyclate Hydrate}} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 1-propanol (1 in 2000).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 3.2 mm in internal diameter and about 1.5 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (0.0075 μm in average pore size, 500 ~ 600 m^2/g in specific surface area 150 ~ 180 μm in particle diameter)

Column temperature: A contant temperature of about 135 ºC.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 5 minutes.

System suitability

System performance: When the procedure is run with 1 μL of th standard solution under the above operating conditions, the resolution between the peaks of ethanol and the internal standard is not less than 2.0.

System repeatability: When the test is repeated 5 times with 1 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethanol with respect to the peak area of the internal standard is not more than 2.0 %.

(2) ***Heavy metals***—Proceed with 1.0 g of Doxycycline Hyclate Hydrate according to Method 2 and perform the test. Prepare the control solution with 5.0 mL of standard lead solution (not more than 50 ppm).

(3) ***Related substances***—Weigh accurately about 120 mg (potency) each of Doxycycline Hyclate Hydrate and Doxycycline RS, dissolve each in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL and use these solutions as the test solution and standard solution (1). Separately, weigh an appropriate amount of Methacycline Hydrochloride RS and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution containing 1.2 mg per mL. Pipet 2.0 mL of this solution and 2.0 mL of standard solution (1), dissolve in 0.01 mol/L hydrochloric acid TS to make 100 mL of a solution containing 0.024 mg of doxycycline and methacycline hydrochloride per mL, and use this solution as standard solution (2). Perform the test with exactly 20 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography according to the following operating conditions, and determine the peak area of methacycline obtained from standard solution (2), A_M, and that obtained from the test solution, A_U. Determine the peak area of doxycycline obtained from standard solution (2), A_S, and the peak area of each related substance other than methacycline obtained from the test solution, A_i. The content of methacycline is not more than 2.0 %, the content of each related substance eluted before the methacycline peak is not more than 0.5 %, the content of 6-epidoxycycline is not more than 2.0 %, and the content of each related substance eluted after the doxycycline peak is not more than 0.5 %.

$$\text{Amount (\%) of methacycline} = \frac{C_M}{W} \times \frac{A_U}{A_M} \times 10000$$

$$\text{Amount (\%) of each related substance other than methacycline} = \frac{C_S}{W} \times \frac{A_i}{A_S} \times 10000$$

C_M: Concentration (mg/mL) of methacycline hydrochloride in standard solution (2)

W: Weight [mg (potency)] of Doxycylcine Hyclate

Hydrate taken

C_S: Concentration [mg (potency)/mL] of doxycycline in standard solution (2)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 60 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutyl ammonium hydrogen sulfate and 0.40 g of disodium dihydrogen ethylenediaminetetraacetate in 850 mL of water. To this solution, add 60 g of *t*-butyl alcohol, add water to make 1000 mL and adjust the pH to 8.0 with 1 mol/L sodium hydroxide. If necessary, increase the resolution between the peaks of related substances and doxycycline and the retention time of doxycycline by reducing the proportion of *t*-butyl alcohol.

Flow rate: About 1.0 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the relative retention times of 4-epidoxycycline, 6-epidoxycycline and doxycycline are about 0.4, 0.7 and 1.0, respectively, the resolution between the peaks of 4-epidoxycycline and doxycycline is not less than 3.0 and the symmetry factor of doxycycline is not more than 2.0.

System repeatability: When the test is repeated 6 times with a solution prepared by dissolving 12 mg of Doxycycline RS in 10 mL of 0.01 mol/L hydrochloric acid, the relative standard deviation is not more than 2.0 %.

Time span of measurement: About 1.7 times as long as the retention time of doxycycline

System suitability solution—Weigh an appropriate amount of Doxycycline RS and dissolve in 0.01 mol/L hydrochloric acid to make a solution containing 6 mg of doxycycline per mL. Take 5 mL of this solution, heat over a steam-bath for 60 minutes and evaporate to dryness on a hot plate, taking care not to burn the residue. Dissolve the residue in 0.01 mol/L hydrochloric acid to make exactly 25 mL, filter through a membrane filter with pore size of not more than 0.5 μm and use this solution as the system suitability solution. Keep this solution in a refrigerator and use within 14 days.

Water 1.4 ~ 2.8 % (0.3 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.3 % (1 g).

Assay Weigh accurately about 0.120 g (potency) of Doxycycline Hyclate Hydrate, dissolve in 0.01 mol/L hydrochloric acid to make exactly 100 mL, filter through a membrane filter with pore size of not more than 0.5 μm, and use the filtrate as the test solution. Separately, weigh accurately about 12 mg (potency) of Doxycycline RS, add 6 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes to dissolve, make exactly 10 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of doxycycline.

$$\text{Amount [μg (potency)] of doxycycline } (C_{22}H_{24}N_2O_8) = \text{Amount [μg (potency)] of Doxycycline RS} \times \frac{A_T}{A_S} \times 10$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 60 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutyl ammonium hydrogen sulfate and 0.40 g of disodium dihydrogen ethylenediaminetetraacetate in 850 mL of water. To this solution, add 60 g of *t*-butyl alcohol, add water to make 1000 mL and adjust the pH to 8.0 ± 0.1 with 1 mol/L sodium hydroxide. If necessary, increase the resolution between the peaks of related substances and doxycycline and the retention time of doxycycline by reducing the proportion of *t*-butyl alcohol.

Flow rate: 1.0 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the relative retention times of 4-epidoxycycline, 6-epidoxycycline and doxycycline are about 0.4, 0.7 and 1.0, respectively, the resolution between the peaks of 4-epidoxycycline and doxycycline is not less than 3.0 and the symmetry factor of doxycycline is not more than 2.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution, the relative standard deviation is not more than 2.0 %.

System suitability solution—Weigh an appropriate amount of Doxycycline RS and dissolve in 0.01 mol/L hydrochloric acid to make a solution containing 6 mg of doxycycline per mL. Take 5 mL of this solution, heat over a steam-bath for 60 minutes and evaporate to

dryness on a hot plate, taking care not to burn the residue. Dissolve the residue in 0.01 mol/L hydrochloric acid to make exactly 25 mL, filter through a membrane filter with pore size of not more than 0.5 μm and use this solution as the system suitability solution. Keep this solution in a refrigerator and use within 14 days.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Doxycycline Hyclate Tablets

Doxycycline Hyclate Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$: 444.44).

Method of Preparation Prepare as directed under Tablets, with Doxycycline Hyclate Hydrate.

Identification The retention time of the main peak obtained from the test solution under the Assay is the same as that obtained from the standard solution.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Doxycycline Hyclate Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution, with the distance between the bottom edge of the stirring blade and the inside bottom of the flask being maintained at 4.5 ± 0.5 cm during the test. Take the dissolved solution after 90 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Doxocycline RS, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Doxycycline Hyclate Tablets at 90 minutes is not less than 85 % (Q).

Dissolution rate (%) with respect to the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of doxycycline ($C_{22}H_{24}N_2O_8$) in 1 tablet

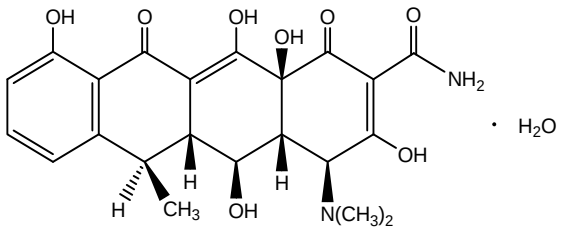
Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Doxycycline Hyclate Hydrate. Weigh and powder not less than 20 Doxycycline Hyclate Tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg (potency) according to the labeled potency, add 75 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes, shake for 15 minutes to dissolve and add 0.01 mol/L hydrochloric acid to make exactly 100 mL. Filter through a membrane filter with pore size of no more than 0.5 μm and use the filtrate as the test solution. Separately, weigh accurately about 10 mg (potency) of Doxycycline RS, add 6 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes to dissolve, make exactly 10 mL and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Doxycycline Hydrate



$C_{22}H_{24}N_2O_8 \cdot H_2O$: 462.45

(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrate [*17086-28-1*]

Doxycycline Hydrate contains not less than 880 μg (potency) and not more than 980 μg (potency) per mg of doxycycline ($C_{22}H_{24}N_2O_8$: 444.44), calculated on the anhydrous basis

Description Doxycycline Hydrate is yellow crystalline powder.

Doxycycline Hydrate is sparingly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in chloroform or in ether.

Doxycycline Hydrate dissolves in dilute acid solution or alkaline solution.

Identification Determine the infrared spectra of Doxycycline Hydrate and Doxycycline RS, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Crystallinity Test It meets the requirement.

pH The pH of a suspension obtained by suspending 0.1 g of Doxycycline Hydrate in 10 mL of water is between 5.0 and 6.5.

Purity (1) ***Heavy metals***—Weigh 0.5 g of Doxycycline Hydrate, mix with 4 mL of a solution of magnesium sulfate heptahydrate in dilute sulfuric acid (1 in 4), heat until a liquid is obtained and evaporate to dryness in a water-bath. Ignite the residue at not exceeding 800 °C to incinerate. Allow to cool and moisten the residue with a small amount of dilute sulfuric acid. Evaporate to dryness, ignite for not more than 2 hours and allow to cool. Extract the residue with two 5 mL volumes of 2 mol/L hydrochloric acid TS, add 0.1 mL of phenolphthalein TS and add ammonia solution (28) until the solution turns pale red. Allow to cool, add acetic acid (100) until the color disappears and add a further 0.5 mL. Filter and wash if necessary. Add water to make 20 mL and use this solution as the test solution. Separately, using 2.5 mL of standard lead solution instead of Doxycycline Hydrate, proceed in the same manner as the preparation of the test solution. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the control solution. Separately, add 2 mL of the test solution to 10 mL of water and use this solution as the blank solution. To 12 mL each of the test solution, the standard solution and the blank solution, add 2 mL of acetate buffer, pH 3.5, mix, add 1.2 mL of thioacetamide TS, mix immediately and allow to stand for 2 minutes: the brown color of the test solution is not more intense than that of the control solution.

System suitability: The control solution shows a faint brown color compared to the blank solution. Add 2.5 mL of standard lead solution to the test solution. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the system suitability solution. The system suitability solution is more intense than or has the same intensity as the control solution.

(2) ***Related substances***—Weigh accurately about 55 mg of Doxycycline Hydrate, dissolve in 12 mL of 0.1 mol/L hydrochloric acid, add 0.01 mol/L hydrochloric acid to make exactly 50 mL and use this solution as the test solution. Weigh accurately a suitable amount of Methacycline Hydrochloride RS, dissolve in 0.01 mol/L hydrochloric acid to make a solution containing 1.2 mg per mL and use this solution as the methacycline standard stock solution. Separately, weigh accurately about 12 mg of Doxycycline RS, dissolve in 0.01 mol/L hydrochloric acid to make exactly 10 mL and use this solution as standard solution (1). Pipet 2.0 mL of standard solution (1) and 2.0 mL of the methacycline standard stock solution, add 0.01 mol/L hydrochloric acid to make exactly 100 mL and use this solution as standard solution (2). Perform the test with 20 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography under the following operating conditions and determine the peak area of methacycline obtained from standard solution (2), A_M, and from the test solution, A_U. Determine the peak area of doxycycline obtained from standard solution (2), A_S, and the peak area of each related substance other than methacycline obtained from the test solution, A_i. The content of methacycline is not more than 2.0 %, the content of related substances eluted before methacycline is not more than 0.5 %, the content of 6-epidoxycycline is not more than 2.0 %, and the content of related substances eluted after the doxycycline peak is not more than 0.5 %.

$$\text{Amount (\%) of methacycline} = \frac{C_M}{W} \times \frac{A_U}{A_M} \times 5000$$

$$\text{Amount (\%) of each related substance other than methacycline} = \frac{C_S}{W} \times \frac{A_i}{A_S} \times 5000$$

C_M: Concentration (mg/mL) of methacycline hydrochloride in standard solution (2)

W: Weight (mg) of Doxycycline Hydrate taken

C_S: Concentration (mg/mL) of doxycycline in standard solution (2)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 60 °C

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutyl ammonium hydrogen sulfate and 0.40 g of disodium dihydrogen ethylenediaminetetraacetate in 850 mL of water. To this solution, add 60 g of *t*-butyl alcohol, add water to make exactly 1000 mL and adjust the pH to between 7.0 and 9.0 with 1 mol/L sodium hydroxide.

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the relative retention times of 4-epidoxycycline, methacycline, 6-epidoxycycline and doxycycline are 0.4, 0.6, 0.7 and 1.0, respectively and the resolution between the peaks of 4-epidoxycycline and doxycycline is not less than 3.0 with the symmetry factor of the peaks being not more than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 2.0 %.

Time span of measurement: About 1.7 times as long as the retention time of doxycycline

System suitability solution—Dissolve Doxycycline RS in 0.01 mol/L hydrochloric acid to make a solution containing 6 mg per mL. Pipet 5 mL of this solution, warm in a water-bath for 60 minutes and evaporate to dryness on a hot plate, taking care not to burn the residue. Dissolve the residue in 0.01 mol/L hydrochloric acid to make exactly 25 mL and use this solution as the system suitability solution. This solution contains 4-epidoxycycline, 6-epidoxycycline and doxycycline. Keep this solution in a refrigerator and use within 14 days.

Water 3.6 ~ 4.6 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Doxycycline Hydrate, equivalent to about 55 mg (potency), dissolve in 12 mL of 0.1 mol/L hydrochloric acid by shaking, add 0.01 mol/L hydrochloric acid to make exactly 50 mL, filter through a filter with pore size of not more than 0.5 μm, and use the filtrate as the test solution. Separately, weigh accurately about 11 mg (potency) of Doxycycline RS, add 6 mL of 0.01 mol/L hydrochloric acid, sonicate for 5 minutes to dissolve, make exactly 10 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography and determine the peak areas, A_T and A_S, of doxycycline in each solution.

$$\text{Amount [μg (potency)] of doxycycline } (C_{22}H_{24}N_2O_8) = \text{Amount [μg (potency)] of doxycycline RS} \times \frac{A_T}{A_S}$$

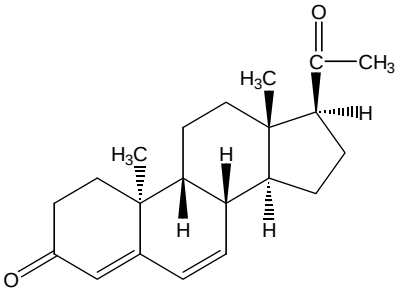
Operating conditions

Proceed as directed in the Assay under Doxycycline Hyclate Hydrate.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Dydrogesterone



$C_{21}H_{28}O_2$: 312.45

(8*S*,9*R*,10*S*,13*S*,14*S*,17*S*)-17-Acetyl-10,13-dimethyl-1,2,8,9,11,12,14,15,16,17-decahydrocyclopenta[a] phenanthren-3-one [*152-62-5*]

Dydrogesterone when dried, contains not less than 98.0 % and not more than 102.0 % of dydrogesterone ($C_{21}H_{28}O_2$).

Description Dydrogesterone appears as white to pale yellow crystals or crystalline powder and is odorless. Dydrogesterone is freely soluble in chloroform, soluble in acetonitrile, sparingly soluble in methanol or in ethanol (95), slightly soluble in ether and practically insoluble in water.

Identification (1) Take 5 mg of Dydrogesterone, add 5 mL of 4-methoxybenzaldehyde-acetic acid TS and 2 to 3 drops of sulfuric acid and heat in a water-bath for 2 minutes: an orange color is observed .

(2) Determine the absorption spectra of solutions of Dydrogesterone and Dydrogesterone RS in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Dydrogesterone and Dydrogesterone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: - 47 ~ - 50° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting Point 167 ~ 171 °C

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Dydrogesterone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 10 mg of Dydrogesterone in 200 mL of the mobile phase and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of dydrogesterone from the test solution is not larger than that of dydrogesterone from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in

internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of water, ethanol (95) and acetonitrile (53 : 26 : 21).

Flow rate: Adjust the flow rate so that the retention time of Dydrogesterone is about 12 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dydrogesterone obtained from 10 μL of the standard solution is between 5 mm and 10 mm.

Selection of column: Dissolve 1 mg each of Dydrogesterone and progesterone in 20 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of dydrogesterone and progesterone in this order with the resolution between these peaks being not less than 8. Wavelength is 265 nm.

Time span of measurement: About twice as long as the retention time of dydrogesterone after the solvent peak.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg of Dydrogesterone, previously dried and dissolve in methanol to make exactly 100 mL. Pipet 1.0 mL of this solution and add methanol to make exactly 100 mL. Determine the absorbance, *A,* of this solution at the wavelength of a maximum absorption at about 286 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of dydrogesterone } (C_{21}H_{28}O_2) = \frac{A}{845} \times 100000$$

Containers and Storage ***Containers***—Tight containers.

Dydrogesterone Tablets

Dydrogesterone Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of dydrogesterone ($C_{21}H_{28}O_2$: 312.45).

Method of Preparation Prepare as directed under Tablets, with Dydrogesterone.

Identification (1) Take a portion of powdered Dydrogesterone Tablets, equivalent to 50 mg of Dydrogesterone according to the labeled amount and add 50 mL of methanol, shake well and filter. Evaporate 5 mL of the filtrate on a water-bath to dryness. Proceed with the residue as directed in the Identification (1) under Dydrogesterone.

(2) Take 1 mL of the filtrate obtained in (1) and add methanol to make exactly 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 284 nm and 288 nm.

Dissolution Test Take 1 tablet of Dydrogesterone Tablets and perform the test using 900 mL of water at 50 revolutions per minute according to Method 2 under the Dissolution Test. After 30 minutes from the start of the test, take 20 mL or more of the dissolved solution and filter. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make *V'* mL so that each mL contains about 50 μg of dydrogesterone, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Dydrogesterone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 24 hours and dissolve in methanol to make exactly 100 mL. Pipet 1.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 296 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Dydrogesterone Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of dydrogesterone } (C_{21}H_{28}O_2) = W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 9$$

W_S: Amount (mg) of Dydrogesterone RS

C: Labeled amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure. Powder 1 tablet of Dydrogesterone Tablets and add methanol to make exactly 100 mL. Shake until the tablet has disintegrated completely and filter through a membrane filter with pore size of not more than 0.45 μm. Discard the first 20 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add methanol to make exactly *V'* mL so that each mL contains about 5 μg of dydrogesterone, and use this solutition as the test solution. Perform the test as directed in the Assay.

$$\text{Amount (mg) of dydrogesterone } (C_{21}H_{28}O_2) = \text{Amount (mg) of Dydrogesterone RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{20}$$

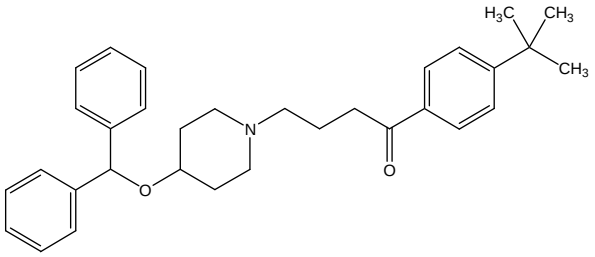
Assay Weigh accurately and powder not less than 20 Dydrogesterone Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of dydrogesterone ($C_{21}H_{28}O_2$), and shake well with 50 mL

of methanol and add methanol to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate, pipet the subsequent 5.0 mL, add methanol to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Dydrogesterone RS previously dried in a desiccator (in vacuum, P_2O_5) for 24 hours, proceed in the same manner as preparation of the test solution and use this solution as this standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 286 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of dydrogesterone } (C_{21}H_{28}O_2) = \text{Amount (mg) of Dydrogestrone RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Ebastine



$C_{32}H_{39}NO_2$: 469.66

4-(4-Benzhydryloxypiperidin-1-yl)-1-(4-tert-butylphenyl)butan-1-one [*90729-43-4*]

Ebastine contains not less than 99.0 % and not more than 101.0 % of ebastine ($C_{32}H_{39}NO_2$), calculated on the anhydrous basis.

Description Ebastine is a white crystalline powder.
Ebastine is very soluble in dichloromethane, slightly soluble in methanol, and practically insoluble in water.
Melting point—About 86 ºC.

Identification Determine the infrared spectra of Ebastine and Ebastine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Sulfates***—Weigh 2.5 g of Ebastine, add 25 ml of dilute nitric acid, reflux with a reflux condenser for 10 minutes, cool and filter. Rinse the filter paper and the residue with adequate amount of water, combine with filtrate, add water to make exactly 50 mL and perform the test using this solution as the test solution. Prepare the control solution mixing 0.53 mL of 0.005 mol/L sulfuric acid VS and 25 mL of dilute nitric acid and adding water to make 50 mL (not more than 0.01 %).

(2) ***Related substances***—Keep the test solution and the standard solution of this test protected from light. Dissolve 0.125 g of Ebastine in mobile phase to make 50 mL and use this solution as the test solution. Dissolve 5.0 mg of Ebastine Related Substance III [4-(Diphenylmethoxy)piperidine] RS and 5.0 mg of Ebastine Related Substance IV {1-[4-(1,1-Dimethylethyl) phenyl]-4-(4-hydroxypiperidine-1-yl)butan-1-one] RS in mobile phase to make exactly 20 mL, pipet 1.0 mL of this solution, add mobile phase to make exactly 100 mL and use this solution as the standard solution (1). To 1.0 mL of the test solution add mobile phase to make exactly 100 mL, pipet 1.0 mL of this solution, add mobile phase to make exactly 10 mL and use this solution as the standard solution (2). Perform the test with exactly 10 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: any peak area of related substances I, II, III, IV, V, VI, and VII in the chromatogram obtained with the test solution is not more than the area of the principal peak with the standard solution (2) (0.1 %), the peak area of the other related substances are not more than that of the principal peak from the standard solution (2) (0.1 %). total area of all related substances is not more than 4 times the area of the principal peak from the standard solution (2) (0.4 %). Disregard the peak being not more than 0.5 times the area of the principal peak with the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with nitrile silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer and acetonitrile (65 : 35). Adjust the percentage of acetonitrile so that the retention time of ebastine is about 110 minutes.

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the test solution under the above operating condition, relative retention time of related substances I, II, III, IV, V, VI, and VII with reference to ebastine are 0.04, 0.05, 0.20, 0.22, 0.42, 0.57 and 1.14, respectively. When the procedure is run with 10 μL of the standard solution (1) under the above operating condition, the resolution between the peaks of related substance IV and related substance III is not less than 2.0.

Time span of measurement: About 1.4 times as long as the retention time of ebastine.

Phosphate buffer—Adjust pH of 0.06 w/v % phosphoric acid with 4 w/v % sodium hydroxide to pH 5.0.

Water Not more than 0.5 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1.0 g).

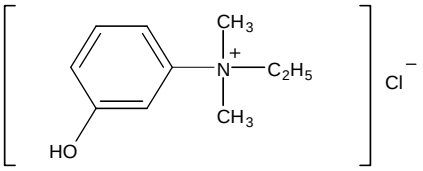
Assay Weigh accurately about 0.35 g of Ebastine, add 50 ml of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.97 mg of $C_{32}H_{39}NO_2$.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Edrophonium Chloride



$C_{10}H_{16}ClNO$: 201.69

N-Ethyl-3-hydroxy-*N,N*-dimethylbenzenaminium chloride [*116-38-1*]

Edrophonium Chloride, when dried, contains not less than 98.0 % and not more than 101.0 % of edrophonium chloride ($C_{10}H_{16}ClNO$).

Description Edrophonium Chloride appears as white crystals or crystalline powder and is odorless.
Edrophonium Chloride is very soluble in water, freely soluble in ethanol (95) or in acetic acid (100), and practically insoluble in acetic anhydride or in ether.
Edrophonium Chloride is hygroscopic.
Edrophonium Chloride is gradually colored by light.

Identification (1) Take 5 mL of a solution of Edrophonium Chloride (1 in 100) and add 1 drop of iron (III) chloride TS: a pale red-purple color is observed.

(2) Determine absorption spectra of solutions of Edrophonium Chloride and Edrophonium Chloride RS, in 0.1 mol/L hydrochloric acid TS (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Edrophonium Chloride (1 in 50) responds to the Qualitative Tests for chloride.

Melting Point 166 ~ 171 ºC (with decomposition).

pH Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Edrophonium Chloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Edrophonium Chloride according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.50 g of Edrophonium Chloride in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution and add ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add ethanol (95) to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (16 : 4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than that from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, in vacuum, P_2O_5, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

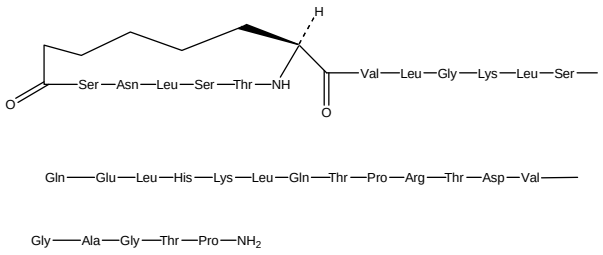
Assay Weigh accurately about 0.2 g of Edrophonium Chloride, previously dried, and dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3). Titrate with 0.1 mol/L perchloric acid VS (potentiometic titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.169 mg of $C_{10}H_{16}ClNO$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Elcatonin



$C_{148}H_{244}N_{42}O_{47}$: 3363.77

N-{[(3*S*,6*S*,9*S*,12*S*)-3-Amino-6-(2-amino-2-oxoethyl)-15-[(1*R*)-1-hydroxyethyl]-12-(hydroxymethyl)-9-isobutyl-4,7,10,13,16,24-hexaoxo-1-oxa-5,8,11,14,17-pentaazacyclotetra-cosan-18-yl]carbonyl}-L-valyl-L-leucylglycyl-L-lysyl-L-leucyl-L-seryl-L-glutaminyl-L-α-glutamyl-L-leucyl-L-histidyl-L-lysyl-L-leucyl-L-glutaminyl-L-threonyl-L-tyrosyl-L-prolyl-L-arginyl-L-threonyl-L-α-aspartyl-L-valylglycyl-L- alanylglycyl-L-threonyl-L-prolinamide [*60731-46-6*]

Elcatonin contains not less than 5000 Elcatonin Units and not more than 7000 Elcatonin Units per 1 mg of peptide, calculated on the dehydrated and de-acetic acid bases.

Description Elcatonin is a white powder.
Elcatonin is very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetonitrile.
Elcatonin is hygroscopic.
The pH of its solution (1 in 500) is between 4.5 and 7.0.

Identification Dissolve 5 mg each of Elcatonin and Elcatonin RS in 5 mL of water. Determine the absorption spectra of both solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent Amino Acids Put about 1 mg of Elcatonin in a test tube for hydrolysis, add phenol-hydrochloric acid TS to dissolve, replace the air inside with Nitrogen, seal the tube under reduced pressure, and heat at 110 ± 2 ºC for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in 1 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the test solution. Separately, weigh exactly 1.33 mg of L-aspartic acid, 1.19 mg of L-threonine, 1.05 mg of L-serine, 1.47 mg of L-glutamic acid, 1.15 mg of L-proline, 0.75 mg of glycine, 0.89 mg of L-alanine, 1.17 mg of L-valine, 1.89 mg of L-tyrosine, 1.83 mg of L-lysine hydrochloride, 2.10 mg of L-histidine hydrochloride monohydrate and 2.11 mg of L-arginine hydrochloride, dissolve them in 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions: 14 peaks of amino acids appear on the chromatogram obtained from the test solution, and their respective molar ratios against alanine are 1.7 ~ 2.2 for aspartic acid, 3.5 ~ 4.2 for threonine, 2.4 ~ 3.0 for serine, 2.7 ~ 3.2 for glutamic acid, 1.7 ~ 2.2 for proline, 2.7 ~ 3.2 for glycine, 1.6 ~ 2.2 for valine, 0.8 ~ 1.2 for 2-aminosuberic acid, 4.5 ~ 5.2 for leucine, 0.7 ~ 1.2 for tyrosine, 1.7 ~ 2.2 for lysine, 0.8 ~ 1.2 for histidine and 0.7 ~ 1.2 for arginine.

Operating conditions

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 μm in particle diameter).

Column temperature: Varied between 50 ºC and 65 ºC.

Chemical reaction vessel temperature: A constant temperature of about 130 ºC.

Color developing time: About 1 minute.

Mobile phase: Buffer solutions A, B, C and D, with sodium ion concentrations of 0.10 mol/L, 0.135 mol/L, 1.26 mol/L and 0.20 mol/L, respectively. The ion concentration of the mobile phase is changed stepwise from 0.10 mol/L to 1.26 mol/L by using these buffer solutions.

Components of buffer solutions (g)

Buffer solution	A	B	C	D
Citric acid	8.85	7.72	6.10	-
Sodium citrate	3.87	10.05	26.67	-
Sodium hydroxide	-	-	2.50	8.00
Sodium chloride	3.54	1.87	54.35	-
Ethanol	60.0 mL	-	-	60.0 mL
Thiodiglycol	5.0 mL	5.0 mL	-	-
Purified water	q.s.	q.s.	q.s.	q.s.
Total amount	1000 mL	1000 mL	1000 mL	1000 mL

Reaction reagent: Mix 407 g of lithium acetate dehydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for about 20 minutes while passing Nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol, add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for about 20 minutes while passing Nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: Adjust the flow rate so

that the retention time of arginine is about 75 minutes.

Flow rate of reaction reagent: About 0.2 mL per minute.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column from which aspartic acid, threonine, serine, glutamic acid, praline, glycine, alanine, valine, 2-aminosuberic acid, leucine, tyrosine, lysine, histidine and arginine are eluted in this order, with complete separation of each peak.

Purity (1) ***Acetic acid***—Weigh accurately about 3 – 6 mg of Elcatonin quickly under conditions of 25 ± 2 °C and 50 ± 5 % relative humidity, add exactly 1 mL of the internal standard solution to dissolve it, and use this solution as the test solution. Separately, weigh accurately about 0.5 g of acetic acid (100), and add the internal standard solution to make exactly 100 mL. Pipet 5 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not more than 7.0 %.

$$\text{Amount (\%) of acetic acid } (CH_3COOH) = \frac{W_{ST}}{W_{SA}} \times \frac{Q_T}{Q_S} \times 50$$

W_{ST}: Amount (g) of acetic acid (100)
W_{SA}: Amount (mg) of sample

Internal standard solution—A solution of citric acid (1 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column abut 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 13.2 g of diammonium hydrogen phosphate in 900 mL of water, add phosphoric acid to adjust the pH to 2.5, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, acetic acid and citric acid are eluted in this order with the resolution between their peaks being not less than 2.0.

(2) ***Related substances***—Dissolve 1.0 mg of Elcatonin in 1 mL of a mixture of trifluoroacetic acid TS and acetonitrile (2:1), and use this solution as the test solution. Take exactly 0.3 mL of the test solution, add a mixture of trifluoroacetic acid TS and acetonitrile (2:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than the peak of elcatonin of the test solution is not larger than the peak of elcatonin of the standard solution, and each peak area other than the peak of elcatonin of the test solution is not larger than 1/3 of the peak area of elcatonin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column abut 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of trifluoroacetic acid TS and acetonitrile (change the ratio linearly from 85:15 to 55:45 in 30 minutes).

Flow rate: Adjust the flow rate so that the retention time of elcatonin is about 25 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of elcatonin from 10 μL of the standard solution is between 50mm and 200 mm.

System performance: Dissolve 2 mg of Elcatonin in 200 μL of trypsin TS for test of elcatonin, warm at 37 ºC for 1 hour, then add 1 drop of acetic acid (100), and heat at 95 ºC for 1 minute. To 10 μL of this solution, add 50 μL of the test solution, and mix. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of elcatonin and the peak which appears immediately before the peak of elcatonin is not less than 2.0, and the retention time of elcatonin is about 25 minutes.

Time span of measurement: Continue measurement until the regularly changing baseline of the chromatogram disappears, beginning after the solvent peak.

Water Weigh accurately 1 ~ 3 mg of Elcatonin quickly under conditions of 25 ± 2 ºC and 50 ± 5 % relative humidity, and perform the test as directed in the coulometric titration under the Water Determination: not more than 8.0 %

Nitrogen Content Weigh accurately 15 to 20 mg of Elcatonin quickly under conditions of 25 ± 2 ºC and 50 ± 5 % relative humidity, and perform the test as directed under the Nitrogen Determination: it contains

not less than 16.1 % and not more than 18.7 % of nitrogen (N: 14.01) in the peptide, calculated on the dehydrated and de-acetic acid basis.

Assay (i) Aminals: Select healthy male Sprague-Dawley rats each weighing between 90 g and 110 g. Keep the rats for not less than 3 days before use, providing an appropriate uniform diet and water.

(ii) Diluent for elcatonin: Dissolve 2.72 g of sodium acetate in water to make 200 mL, add 0.2 g of bovine serum albumin, and adjust the pH to 6.0 with glacial acetic acid. Prepare before use.

(iii) Standard solution: Dissolve Elcatonin RS in the diluent for elcatonin to make two standard solutions, one to contain exactly 0.75 Unit in each mL which is designated as the high-dose standard solution, S_H, and the other to contain exactly 0.0375 Unit in each mL which is designated as the low-dose standard solution, S_L.

(iv) Test solution: Weigh accurately 0.5 2.0 mg of Elcatonin quickly under conditions of 25 ± 2 ºC and 50 ± 5 % relative humidity, and dissolve in the diluent for elcatonin to make two test solutions, the high-dose test solution, T_H, which contains the Units per mL equivalent to S_H and the low-dose test solution, T_L, which contains the Units per mL equivalent to S_L.

(v) Deproteinizing solution for elcatonin: Dissolve 160 g of trichloroacetic acid and 30.6 g of strontium chloride in water to make 3600 mL.

(vi) Procedure: Divide the animals into 4 equal groups of not less than 10 animals each. Withhold all food, but not water, for 18 to 24 hours before the injection, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject exactly 0.2 mL each of the standard solutions and the test solutions into the tail vein of each animal as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

At 1 hour after the injection, take a sufficient amount blood sample to perform the test from the carotid artery and vein of each animal under ether anesthesia, centrifuge the blood samples to separate serum, and determine the serum calcium according to the following (vii).

(vii) Serum calcium determination: Take exactly 0.3 mL of the serum, add the deproteinizing solution for elcatonin to make exactly 3 mL, mix well, centrifuge, and use the clear supernatant liquid as the test solution for calcium determination. Separately, pipet 1 L of Standard Calcium Solution for the Atomic Absorption Spectrophotometry, and add a solution of sodium chloride (17 in 2000) to make exactly 10 mL. Pipet 5 mL of this solution, add the deproteinizing solution for elcatonin to make exactly 50 mL, and use this solution as the standard solution for calcium determination. Determine the absorbances, A_T and A_S, of the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions. Determine the absorbance, A_0, of a solution obtained in the same manner used for preparation of the standard solution, but with 1 mL of water instead of the standard solution.

$$\text{Amount (mg) of calcium in 100 mL of the serum} = 0.01 \times \frac{A_T - \mathrm{A}_0}{\mathrm{A}_S - \mathrm{A}_0} \times 10 \times 100$$

Gas: Combustible gas—Dissolved acetylene – Air
Lamp: Calcium hollow-cathode lamp
Wavelength: 422.7 nm

(viii) Calculation: Amounts of calcium in 100 mL of the serum obtained with S_H, S_L, T_H and T_L in (vii) are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up individual y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Units per mg of peptide, calculated on the dehydrated and de-acetic acid basis
= antilog $M \times$ (units per mL of S_H) $\times$ (b/a)

$$M = 0.3010 \times \frac{Y_a}{Y_b}$$
$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$
$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Amount (mg) of the sample $\times$
$$\{100 - \frac{[\text{water content (\%)} + \text{acetic acid content (\%)}]}{100}\}$$

b: Total volume (mL) of the high-dose test solution prepared by dissolving the sample with diluent for elcatonin

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L (P = 0.95) by use of the following equation: L should be not more than 0.20. If F' exceeds F, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F and L is not more than 0.20.

$$F' = \frac{(-Y_1 + Y_2 + Y_3 - Y_4)^2}{4fs^2}$$

f: Number of the animals of each group.

$$s^2 = \frac{\sum y^2 - \frac{\mathrm{y}}{\mathrm{f}}}{\mathrm{n}}$$

$\sum y^2$: The sum of squares of y_1, y_2, y_3 and y_4 in each group.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f-1)$$

$$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

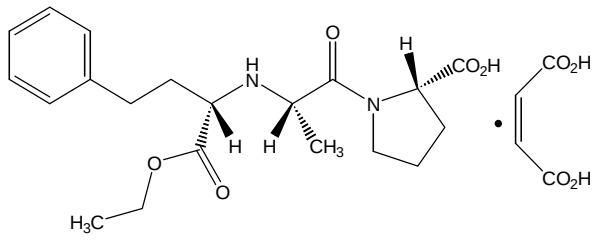
t^2: Value shown in the following table against n used to calculate s^2.

n	$t^2 = F$	n	$t^2 = F$	n	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 8 ºC.

Enalapril Maleate



$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$: 492.52

(2*S*)-1-[(2*S*)-2-{[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-but-2-enedioate [*76095-16-4*]

Enalapril Maleate contains not less than 98.0 % and not more than 102.0 % of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$), calculated on the dried basis.

Description Enalapril Maleate appears as white crystals or crystalline powder.

Enalapril Maleate is freely soluble in methanol, sparingly soluble in water or in ethanol (99.5), and slightly soluble in acetonitrile.

Melting point—About 145 °C (with decomposition).

Identification (1) To 20 mg of Enalapril Maleate add 1 mol/L hydrochloric acid TS, shake, add 5 mL of ether, and shake for 5 minutes. Take 3 mL of the upper layer, distill the ether in a water bath, and add 5 mL of water to the residue while shaking. Add 1 drop of potassium permanganate TS: the red color of the test solution disappears immediately.

(2) Determine the infrared spectra of Enalapril Maleate and Enalapril Maleate RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Specific Optical Rotation $[\alpha]_D^{25}$: -41.0 ~ -43.5° (after drying, 0.1 g, methanol, 10 mL, 100 mm) .

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Enalapril Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 30 mg of Enalapril Maleate, dissolve in a mixture of pH 2.5 phosphate buffer solution and acetonitrile (95 : 5) to make exactly 100 mL. and use this solution as the test solution. Separately, weigh accurately about 30 mg of Enalapril Maleate RS, dissolve in a mixture of pH 2.5 phosphate buffer solution and acetonitrile (95 : 5) to make exactly 100 mL Pipet 1 mL of this solution, add a mixture of pH 2.5 phosphate buffer solution and acetonitrile (95 : 5) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 50 µL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area by the automatic integration method, calculate each amount of related substances: among peaks other than the principal peak one related substance is not more than 1.0 %, another related substance is not more than 0.3 % and the total area of peaks of the related substances is not more than 2.0 %.

$$\text{Amount (\%) of related substaced} = 100 \times \frac{C_S}{C_i} \times \frac{A_T}{A_S}$$

C_S: Concentration of the standard solution (mg/mL)

C_i: Concentration of Enalapril Maleate in the test solution (mg/mL)

A_T: Peak area of each related substances in test solution

A_S: Peak area of each related substances in standard solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Loss on Drying Not more than 1.0 % (at a pressure not exceeding 0.67 kPa, 60 ºC, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 30 mg of Enalapril Maleate, dissolve in a mixture of pH 2.5 phosphate buffer solution and acetonitrile (95 : 5) to make exactly 100 mL, and use this solution as the test solution. Separately, dissolve about 30 mg, accurately weighed, of Enalapril Maleate in a mixture of pH 2.5 phosphate buffer solution and acetonitrile (95 : 5) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, A_T and A_S, of the peak area of Enalapril Maleate to that of the internal standard in each solution.

$$\text{Amount (mg) of enalapril maleate } (C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4) = \text{Amount (mg) of Enalapril Maleate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Ditector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, 4.1 mm in internal diameter and about 15 cm in length, packed with styrenedivinyl benzene for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: 70 ºC

Flow rate: 1.5mL/minute.

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of pH 6.8 phosphate buffer solution and acetonitrile (19 : 1)

Mobile phase B: A mixture of acetonitrile and pH 6.8 phosphate buffer solution (33 : 17)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	95	5
0-20	95→40	5→60
20-25	40	60
25-26	40→95	60→5
26-30	95	5

System suitability

System performance: Mix 1 mL of Enalapril diketopiperazine solution and 50 mL of the standard solution. When the procedure is run with 50 μL of this solution, as directed under the above operating conditions, Enalapril and enalapril diketopiperazine are eluted in this order with the resolution between their peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak area is not more than 1.0 %.

pH 6.8 phosphate buffer solution: Dissolve 2.8 g of sodium dihydrogen phosphate dihydrate in about 900 mL of water in a 1000mL volumetric flask. Adjust with phosphoric acid to a pH of about 6.8, dilute with water to volume, and mix.

pH 2.5 phosphate buffer solution: Dissolve 2.8 g of sodium dihydrogen phosphate dihydrate in about 900 mL of water in a 1000mL volumetric flask. Adjust with phosphoric acid to a pH of about 2.5, dilute with water to volume, and mix.

Enalapril diketopiperazine solution: Place about 20 mg of Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting. Heat for about 5 minutes to 10 minutes until the solid is melted. Immediately remove the beaker from the hot plate, and allow to cool. Add 50 ml of acetonitrile, and sonicate for a few minutes to dissolve. The solution typically contains, in each mL, between 0.2 mg and 0.4 mg of enalapril diketopiperazine.

Containers and Storage ***Containers***—Well-closed containers.

Enalapril Maleate Tablets

Enalapril Maleate Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$: 492.52).

Method of Preparation Prepare as directed under Tablets, with Enalapril Maleate.

Identification (1) To an amount of powdered Enalapril Maleate Tablets, equivalent to 50 mg of Enalapril Maleate according to the labeled amount, add 20 mL of methanol, and shake well. Centrifuge this solution, and use the clear supernatant liquid as the test solution. Separately, dissolve 25 mg of Enalapril Maleate RS in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of water, acetone, 1-butanol, and acetic acid (100) (1 : 1 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the two spots obtained from the test solution show the same R_f value as the two spots obtained from the standard solution.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity ***Related substances***—Use the test solution, standard solution and pH 2.2 phosphate buffer solution prepared as directed in the Assay, and enalapril diketopiperazine solution prepared as directed in the Assay under Enalapril Diketopiperazine Maleate. Pipet 1.0 mL of the standard solution, add pH 2.2 phosphate buffer solution to make exactly 100mL and use this solution as the related substances standard solution. Perform the test with 50 μL of each of the test solution, standard solution, related substances standard solution and buffer solution as directed under Liquid Chromatography according to the operating conditions in the Assay. Determine each peak areas by the automatic integration method, and calculate the amounts of related substances. Measure the peak areas for all of the peak from the test solution, having the peak area not less than 0.1 % of that of enalapril, other than those are observed in the buffer solution.

$$\text{Amount (\%) of anhydrous enlalprilat} = \frac{492.53}{348.39} \times \frac{CV}{N} \times \frac{A_T}{A_S} \times \frac{100}{L}$$

492.53: Molecular weight of enalapril maleate
348.39: Molecular weight of anhydrous enalaprilat
C: Concentration (mg/mL) of enalaprilat in the standard solution.
V: Volume (mL) of the test solution
N: Number of tablets taken for the test
L: Labeled amount (mg) of enalapril maleate in the tablet
A_T: Peak area of enalaprilat obtained from the test solution
A_S: Peak area of enalaprilat obtained from the standard solution

$$\text{Amount (\%) of enalapril ketopiperazine} = \frac{492.53}{358.44} \times \frac{C'V}{N} \times \frac{A_T}{1.25\,A_S} \times \frac{100}{L}$$

492.53: Molecular weight of enalapril maleate
358.44: Molecular weight of enalapril ketopiperazine
C': Concentration (mg/mL) of Enalapril Maleate RS in the related substances standard solution
V: Volume (mL) of the test solution
N: Number of tablets taken for the test
1.25: Peak area of enalapril diketopiperazine relative to that of enalapril
L: Labeled amount (mg) of enalapril maleate in the tablet

$$\text{Amount (\%) of each other related substance} = \frac{C'V}{N} \times \frac{A_T}{A_S} \times \frac{100}{L}$$

A_T: Peak area of each other related substance
C', V, N are defined as the above.

The total amount of all the related substances including enalaprilat and enalapril diketopiperazine is not more than 5.0 %.

Enalaprilat standard solution—Weigh accurately a suitable amount of Enalaprilat RS, dissolve in water to make a solution so that each mL contains 0.4 mg.

Standard solution—Weigh accurately about 20 mg of Enalapril Maleate RS, add 0.5mL of enalaprilate standard solution and 50 mL of buffer solution, shake well to dissolve, add pH 2.2 phosphate buffer solution to make exactly 100mL.

Related substances standard solution—Pipet 1 mL of the standard solution, add pH 2.2 phosphate buffer solution to make exactly 100 mL.

Dissolution Test Perform the test with 1 tablet of Enalapril Maleate Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of pH 6.8 phosphate buffer solution as a dissolution solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent solution as the test solution. Separately, weigh accurately about 10 mg of Enalapril Maleate RS, dissolve in pH 6.8 phosphate buffer solution to make exactly 100 mL and use this solution as the standard solution. Perform the test according to the operating conditions as directed in the Assay under Enalapril Maleate. The dissolution rate of Enalapril Maleate Tablets in 30 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement of the Content Uniformity Test when the test is performed according to the following method.
Take 1 tablet of Enalapril Maleate Tablets, add 50 mL of pH 2.2 phosphate buffer solution, if necessary, sonicate for 15 mintes, shake for 30 minutes, add pH 2.2 phosphate buffer solution to make exactly 100 mL and use this solution as the test solution. Pipet V mL each of this solution, then add buffer solution to make a solution containing 0.1 mg/mL. Separately, weigh accurately about 10 mg of Enalapril Maleate RS, add pH 2.2 phosphate buffer to make exactly 100mL and use this solution as the standard solution**.** Perform the test according to the operating conditions as directed in the Assay under Enalapril Maleate.

$$\text{Amount (mg) of Enalapril Maleate } (C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4) = T \times \frac{C}{D} \times \frac{A_T}{A_S}$$

T: Labeled amount (mg) of enalaptil meleate in 1

tablet

C: Concentration (mg/mL) of the standard solution.

D: Concentration (mg/mL) of enalapril maleate in the test solution.

A_T: Peak are of enalapril maleate in the test solution.

A_S: Peak area of enalapril maleate in the standard solution

pH 2.2 phosphate buffer—Dissolve 1.38g of sodium dihydrogen phosphate dihydrate in about 800 mL of water in a 1000mL volumetric flask. Adjust with phosphoric acid to a pH of about 2.2, dilute with water to volume, and mix.

Assay Weigh accurately and powder not less than 20 Enalapril Maleate Tablets, weigh accurately a portion of the powder, equivalent to about 20 mg of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$), add 50 mL of pH 2.2 phosphate buffer solution. Ultrasonicate for 15 minutes, then shake for 30 minutes, add pH 2.2 phosphate buffer solution to make exactly 100 mL, sonicate for 15 minutes and filter through a membrane filter. Separately, weigh accurately about 20 mg of Enalapril Maleate RS, add 0.5mL of enlalaprilat standard solution and dissolve in 50 mL of pH 2.2 phosphate buffer solution, then sonicate, if necessary, add pH 2.2 phosphate buffer solution to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area, A_T and A_S, of each solution by the automatic integration method

$$\text{Amount (mg) of enalapril maleate } (C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4) = \text{Amount (mg) of Enalapril Maleate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with hydroxypropylsilanized silica gel for liquid chromatography.

Column temperature: 50 ºC

Mobile phase: The mixture of pH 2.2 phosphate buffer solution and acetonitrile (75 : 25).

Flow rate: 2mL/minute.

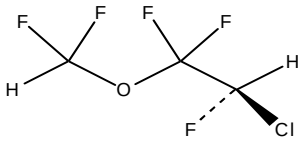
System suitability

System performance: Pipet 0.5mL of Enalapril diketopiperazine solution, add the standard solution to make 25mL. When the procedure is run with 50 μL of this solution, as directed under the above operating conditions, maleic acid, enalaprilat, enalapril, enalapril diketopiperazine are eluted in this order with the resolution between maleic acid and enalaprilat, between enalaprilat and enalapril, and between enalapril and enalapril diketopiperazine being not less than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Enflurane



and enantiomer

$C_3H_2ClF_5O$: 184.49

2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane [*13838-16-9*]

Description Enflurane is a colorless clear liquid.
Enflurane is slightly soluble in water, miscible with ethanol (95) or ether.
Enflurane is volatile and not flammable.
Enflurane has no optical activity.

Boiling point—54 ~ 57 ºC.

Identification (1) Perform the test with 50 μL of Enflurane as directed under the Oxygen Flask Combustion Method to obtain the test solution. The test solution responds to the Qualitative Tests for chloride and fluoride.

(2) Determine the infrared spectra of Enflurane and Enflurane RS, previously dried, as directed in the liquid film method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20} : 1.302 ~ 1.304.

Specific Gravity d_{20}^{20} : 1.520 ~ 1.540.

Purity (1) ***Acidity and alkalinity***—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes to mix, allow to separate completely and use the water layer as the test solution. To 20 mL of the test solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L of sodium hydroxide VS: the color of the solution is violet. To 20 mL of the test solution add 1 drop of bromocresol purple TS and 60 μL of 0.01 mol/L hydrochloric acid: VS the color of the solution is yellow.

(2) ***Chloride***—Weigh 20 g of Enflurane, add 20 mL of water, shake for 5 minutes, allow to separate completely and use water layer. Pipet 10 mL of the

solution and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001 %).

(3) ***Related substances***—Perform the test with 5 μL of Enflurane as directed under Gas Chromatography according to the following conditions, determine each peak area other than the peak of air which appears soon after injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10 %

Operating conditions

Detector: A thermal conductivity detector.

Column: A column about 3 mm in internal diameter and about 3 m in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter), coated with diethylene glycol succinate ester for gas chromatography (5 μm in particle diameter) in the ratio of 20 %.

Column temperature: A constant temperature of about 80 ºC.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of enflurane is about 3 minutes.

System suitability

Test for required detectability: Pipet 1 mL of Enflurane and add 2-propanol to make 100 mL. Pipet 2 mL of this solution, add 10 mL of 2-propanol and use this solution as the solution for system suitability. Pipet 1 mL of this solution and dilute with 2-propanol to exactly 10 mL. Confirm that the peak area of enflurane obtained with 5 μL of this solution is equivalent to 7 to 13 % of that with 5 μL of the solution for system suitability.

System performance: Mix 5 mL each of Enflurane and 2-propanol. When the procedure is run with 5 μL of this solution under the above operating conditions, enflurane, 2-propanol are eluted in this order with the resolution between the peaks is not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of enflurane is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of enflurane beginning after the solvent peak.

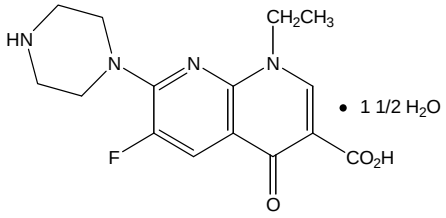
(4) ***Residue on evaporation***—Pipet 65 mL of Enflurane, evaporate on steam bath to dryness and dry the residue at 105 ºC for 1 hour: the amount of residue is not more than 1.0 mg.

Water Not more than 0.1 % (10 g, volumetric titration, direct titration).

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 ºC.

Enoxacin Hydrate



$C_{15}H_{17}FN_4O_3 \cdot 1½H_2O$: 347.34

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid sesquihydrate
[*84294-96-2*]

Enoxacin Hydrate, when dried, contains not less than 98.5 % and not more than 101.0 % of enoxacin ($C_{15}H_{17}FN_4O_3$: 320.32).

Description Enoxacin Hydrate appears as white to pale yellow-brown crystals or crystalline powder.
Enoxacin Hydrate is freely soluble in acetic acid (100), slightly soluble in methanol, very slightly soluble in chloroform, and practically insoluble in water, in ethanol (95) or in ether.
Enoxacin Hydrate dissolves in dilute sodium hydroxide TS.
Enoxacin Hydrate is gradually colored by light.

Identification (1) Place 20 mg of Enoxacin Hydrate and 50 mg of metallic sodium in a test tube and heat gradually to ignition with precaution. After cooling, add 0.5 mL of methanol and then 5 mL of water and heat to boiling. To this solution, add 2 mL of dilute acetic acid and filter: the filtrate responds to the Qualitative Tests (2) for fluoride.

(2) Dissolve 50 mg each of Enoxacin Hydrate and Enoxacin Hydrate RS, in dilute sodium hydroxide TS to make 100 mL each. To each of 1 mL of the solutions, add water to make 100 mL each and determine the absorption spectra as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Enoxacin and Enoxacin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 225 ~ 229 ºC (after drying).

Purity (1) ***Sulfate***—Dissolve 1.0 g of Enoxacin Hydrate in 50 mL of dilute sodium hydroxide TS, shake with 10 mL of dilute hydrochloric acid and centrifuge.

Filter the clear supernatant liquid and to 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS, add 25 mL of dilute sodium hydroxide TS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Enoxacin Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Enoxacin Hydrate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Enoxacin Hydrate in 25 mL of a mixture of chloroform and methanol (7 : 3) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of chloroform and methanol (7 : 3) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than that from the standard solution.

Loss on Drying 7.0 ~ 9.0 % (1 g, 105 ºC, 3 hours).

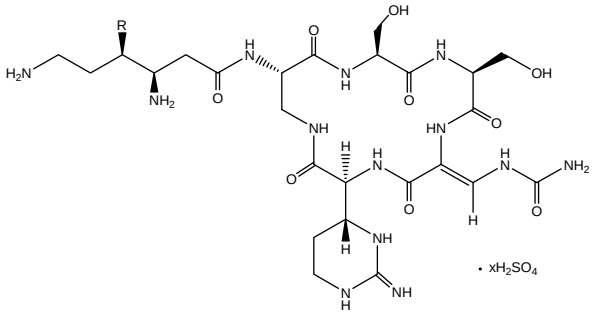
Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.3 g of Enoxacin Hydrate, previously dried, dissolve in 30 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.032 mg of $C_{15}H_{17}FN_4O_3$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Enviomycin Sulfate



Tuberactinomycin N : R = O
Tuberactinomycin O : R = H

Tuberactinomycin N sulfate
$C_{25}H_{43}N_{13}O_{10} \cdot 1\frac{1}{2} : H_2SO_4 : 832.81$

Tuberactinomycin O sulfate
$C_{25}H_{43}N_{13}O_9 \cdot 1\frac{1}{2} : H_2SO_4 : 816.81$

Tuberactinomycin N Sulfate:
3,6-Diamino-*N*-[(3*R*,6*Z*)-3-(2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)-6-[(carbamoylamino)methylidene]-9,12-bis(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-15-yl]-4-hydroxyhexanamide sesquisulfate [*33103-22-9*, Tuberactinomycin N]
Tuberactinomycin O Sulfate:
3,6-Diamino-*N*-[(3*R*,6*Z*)-3-(2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)-6-[(carbamoylamino)methylidene]-9,12-bis(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-15-yl]hexanamide sesquisulfate [*33137-73-4*, Tuberactinomycin O]

Enviomycin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Streptomyces griseoverticillatus var. tuberacticus*.
Enviomycin Sulfate contains not less than 770 μg (potency) per mg of tuberactinomycin ($C_{25}H_{43}N_{13}O_{10}$: 685.69), calculated on the dried basis.

Description Enviomycin Sulfate appears as white powder.
Enviomycin Sulfate is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 5 mL of a solution of Enviomycin Sulfate (1 in 200) add 1.5 mL of sodium hydroxide TS and 1 drop of a solution prepared by adding 0.01 mol/L citric acid TS to 3 mL of copper (II) sulfate TS to make 100 mL: a blue-purple color is produced.

(2) To 2 mL of a solution of Enviomycin Sulfate (1 in 20) add 1 drop of barium chloride TS: a white precipitate is produced.

(3) Determine the absorption spectra of solutions of Enviomycin Sulfate and Enviomycin Sulfate RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: -16 ~ -22° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm)

pH The pH of a solution obtained by dissolving 2.0 g of Enviomycin Sulfate in 20 mL of water is between 5.5 and 7.5.

Absorbance $E_{1cm}^{1\%}$ (268 nm): 280 ~ 360 (10 mg, water, 1000 mL).

Content Ratio Dissolve 0.1 g of Enviomycin Sulfate in water to make 100 mL, and use this solution as the test solution. Perform the test with 3 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_{T1} and A_{T2}, of tuberactinomycin N and tuberactinomycin O, having relative retention time of 1.4 ± 0.4 with respect to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1}+A_{T2})$ is between 0.090 and 0.150.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of ammonium acetate TS, 1,4-dioxane, tetrahydrofuran, water, and ammonia solution (28) (100 : 75 : 50 : 23 : 2)

Flow rate: Adjust the flow rate so that the retention time of tuberactinomycin N is about 9 minutes.

System suitability

System performance: When the procedure is run with 3 μL of the test solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0 %.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Enviomycin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

Loss on Drying Not more than 4.0 % (0.2 g, in vacuum, P_2O_5, 60 °C, 3 hours)

Sterility Test It meets the requirement, when Enviomycin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.33 EU/mg (potency) of enviomycin, when Enviomycin Sulfate is used in a sterile preparation.

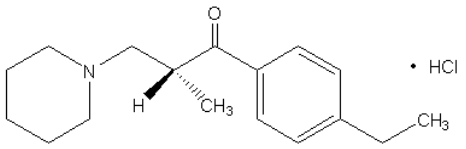
Assay ***The Cylinder-plate method*** (1) Test organism- *Bacillus subtilis* ATCC 6633

(2) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(3) Weigh accurately about 20 mg (potency) of Enviomycin Sulfate, and dissolve in water to make exactly 20 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 400 μg (potency) and 100 μg (potency) and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Enviomycin Sulfate RS, dissolve in water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 10 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Eperisone Hydrochloride



and enantiomer

$C_{17}H_{25}NO \cdot HCl$: 295.85

1-(4-Ethylphenyl)-2-methyl-3-(piperidin-1-yl)propan-1-one hydrochloride [*56839-43-1*]

Eperisone Hydrochloride contains not less than 98.5 % and not more than 101.0 % of eperisone hydrochloride ($C_{17}H_{25}NO \cdot HCl$), calculated on the anhydrous basis.

Description Eperisone Hydrochloride appears as white crystalline powder.
Eperisone Hydrochloride is freely soluble in water, methanol, or in acetic acid (100), and soluble in ethanol (99.5).

Melting point—About 167 °C (with decomposition).
A solution of Eperisone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Eperisone Hydrochloride and Eperisone Hydrochloride RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Eperisone Hydrochloride and Eperisone Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Eperisone Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Eperisone Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Piperidine hydrochloride***—Dissolve 1.0 g of Eperisone Hydrochloride in 20 mL of water, add 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20), and 1.5 mL of ammonia solution (28), and use this solution as the test solution. Separately, to 2.0 mL of a solution of piperidine hydrochloride (1 in 1000) add 18 mL of water, 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20), and 1.5 mL of ammonia solution (28), and use this solution as the standard solution. To the test solution and standard solution add 10 mL each of a mixture of isopropyl ether and carbon disulfide (3 : 1), mix well for 30 seconds, allow to stand for 2 minutes, and compare the colors of the two supernatant liquid layers: the color of the test solution is not more intense than that of the standard solution.

(3) ***Related substances***—Dissolve 0.1 g of Eperisone Hydrochloride in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the total area of the peaks other than eperisone from the test solution is not larger than 1/5 times the peak area of eperisone from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: A mixture of methanol, 0.0375 mol/L sodium 1-decanesulfonate solution, and perchloric acid (600 : 400 : 1)

Flow rate: Adjust the flow rate so that the retention time of eperisone is 17 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of eperisone obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of eperisone are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak areas of eperisone is not more than 3.0 %.

Time span of measurement: About 2 times as long as the retention time of eperisone

Sodium 1-decanesulfonate solution, 0.0375 mol/L—Dissolve 3.665 g of sodium 1-decanesulfonate in 400 mL of water.

Water Not more than 0.2 % (0.1 g, coulometric titration)

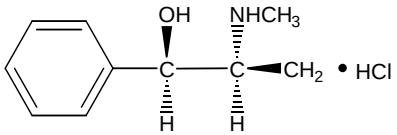
Residue on Ignition Not more than 0.2 % (1 g)

Assay Weigh accurately about 0.6 g of Eperisone Hydrochloride, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank titration and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 29.59 mg of $C_{17}H_{25}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Ephedrine Hydrochloride



$C_{10}H_{15}NO \cdot HCl$: 201.69

(1*R*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride [*50-98-6*]

Ephedrine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Description Ephedrine Hydrochloride appears as white crystals or crystalline powder.
Ephedrine Hydrochloride is freely soluble in water, soluble in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride or acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Ephedrine Hydrochloride and Ephedrine Hydrochloride RS, respectively, in water (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ephedrine Hydrochloride and Ephedrine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Ephedrine Hydrochloride (1 in 15) responds to the Qualitative Tests for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: - 33.0 ~ - 36.0° (after drying, 1 g, water, 20 mL, 100 mm).

Melting Point 218 ~ 222 ºC.

pH A solution of Ephedrine Hydrochloride (1 in 20) is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Ephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Acid or alkali***—Weigh accurately 1.0 g of Ephedrine Hydrochloride, dissolve in 20 mL of water, and add 1 drop of methyl red TS. If the solution is yellow, add 0.01 mol/L sulfuric acid until the solution shows a red color: not more than 0.10 mL is consumed. If the solution is pink, add 0.02 mol/L sodium hydroxide VS until the solution shows a yellow color: not more than 0.20 mL is consumed.

(3) ***Sulfate***—Dissolve 50 mg of Ephedrine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS and allow to stand for 10 minutes: no turbidity is produced.

(4) ***Heavy Metals***—Proceed with 1.0 g of Ephedrine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) ***Related Substance***—Dissolve 50 mg of Ephedrine Hydrochloride in 50 mL of mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following condition and calculate the areas of each peak by the automatic integration method: the total area of all peaks other than principal peak from the test solution is not greater than that of principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 ºC.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 128), acetonitrile and phosphoric acid (640 : 360 : 1)

Flow rate: Adjust the flow rate so that the retention time of ephedrine is about 14 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add mobile phase to make 20 mL. Confirm that the peak area of ephedrine obtained from 10 μL of this solution is equivalent to 4 to 6 % of that from the standard solution.

System performance: Dissolve 1 mg of Ephedrine Hydrochloride RS and 4 mg of atropine sulfate in 100 mL of diluted methanol (1 in 2). When the procedure is run with 10 μL of this solution as directed under the above operating conditions, ephedrine and atropine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak areas of ephedrine is not more than 2.0 %.

Time span of measurement: About three times as long as the retention time of ephedrine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Ephedrine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) by warming. Cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, endpoint detection method in titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.169 mg of $C_{10}H_{15}NO{\cdot}HCl$

Containers and Storage ***Containers***—Well-closed containers.

Ephedrine Hydrochloride Injection

Ephedrine Hydrochloride Injection is an aqueous solution for injection. Ephedrine Hydrochloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO{\cdot}HCl$: 201.69).

Method of Preparation Prepare as directed under Injections, with Ephedrine Hydrochloride.

Description Ephedrine Hydrochloride Injection is a clear, colorless liquid.

pH—4.5 ~ 6.5.

Identification Take a volume of Ephedrine Hydrochloride Injection, equivalent to 50 mg of Ephedrine Hydrochloride according to the labeled amount, add water to make 100 mL and the determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 249 and 253 nm, between 255nm and 259 nm and between 261 nm and 265 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 7.5 EU/mg of Ephedrine Hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Take an exact volume of Ephedrine Hydrochloride Injection, equivalent to about 40 mg of ephedrine hydrochloride ($C_{10}H_{15}NO{\cdot}HCl$) according to the labeled amount, add exactly 10 mL of the internal standard solution and water to make 200 mL and use this solution as the test solution. Separately, weigh accurately about 40 mg of Ephedrine Hydrochloride RS, previously dried at 105 ºC for 3 hours, add exactly 10 mL of internal standard solution and water to make 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following condition and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard of each solution.

$$\text{Amount (mg) of ephedrine hydrochloride } (C_{10}H_{15}NO{\cdot}HCl) = \text{Amount (mg) of Ephedrine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution as directed under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

10 % Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder contains not less than 9.3 % and not more than 10.7 % of ephedrine hydrochloride ($C_{10}H_{15}NO{\cdot}HCl$: 201.69).

Method of Preparation

Ephedrine Hydrochloride	100 g
Starch, Lactose Hydrate	a sufficient quantity or their mixture
Total	1000 g

Prepare as directed under Powders, with the above in-

gredients.

Identification Take 0.5 g of 10 % Ephedrine Hydrochloride Powder, add 100 mL of water, shake for 20 minutes and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm and between 261 nm and 265 nm.

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Weigh accurately about 0.4 g of 10 % Ephedrine Hydrochloride Powder, add 150 mL of water and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge and use the supernatant as the test solution. Separately, weigh accurately about 40 mg of Ephedrine Hydrochloride RS, previously dried at 105 ºC for 3 hours, add exactly 10 mL of internal standard solution and water to make 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following condition and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard of each solution.

$$\text{Amount (mg) of ephedrine hydrochloride } (C_{10}H_{15}NO\cdot HCl) = \text{Amount (mg) of Ephedrine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution as directed under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Ephedrine Hydrochloride Tablets

Ephedrine Hydrochloride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO\cdot HCl$: 201.69).

Method of Preparation Prepare as directed under Tablets, with Ephedrine Hydrochloride.

Identification Take an amount of powdered Ephedrine Hydrochloride Tablets, equivalent to 50 mg of Ephedrine Hydrochloride according to the labeled amount, add 100 mL of water, shake for 20 minutes and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm and between 261 nm and 265 nm.

Dissolution Test Perform the test with 1 tablet of Ephedrine Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 28 mg of Ephedrine Hydrochloride RS, previously dried at 105 °C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of ephedrine. The dissolution rate of Ephedrine Hydrochloride Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of ephedrine } (C_{10}H_{15}NO\cdot HCl) = \text{Amount (mg) of Ephedrine RS taken} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90$$

C: Labeled amount (mg) of ephedrine ($C_{10}H_{15}NO\cdot HCl$) in 1 tablet

Operating conditions

Proceed as directed in the operating conditions in the Purity (5) Related substances under Ephedrine Hydrochloride.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ephedrine are not less than 10000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ephedrine is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 tablets of Ephedrine Hydrochloride Tablets. Weigh accurately an amount of powder, equivalent to about 40 mg of Ephedrine Hydrochloride, add 150 mL of water and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 40 mg of Ephedrine Hydrochloride RS, previously dried at 105 ºC for 3 hours, add exactly 10 mL of internal standard solution and water to make 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following condition and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard of each solution.

$$\text{Amount (mg) of ephedrine hydrochloride } (C_{10}H_{15}NO\cdot HCl) = \text{Amount (mg) of Ephedrine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

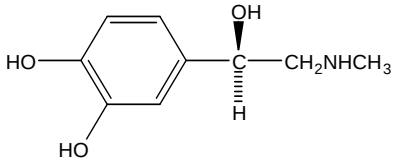
System suitability

System performance: When the procedure is run with 10 μL of the standard solution as directed under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Epinephrine



Adrenaline
Epirenamine $C_9H_{13}NO_3$: 183.20

4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol [*51-43-4*]

Epinephrine, when dried, contains not less than 98.0 % and not more than 101.0 % of epinephrine ($C_9H_{13}NO_3$: 183.20).

Description Epinephrine is a white to grayish white, crystalline powder and odorless.
Epinephrine is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in methanol, ethanol (95) or ether.
Epinephrine dissolves in dilute hydrochloric acid.
Epinephrine gradually changes to brown in color by air and by light.

Identification (1) Dissolve 10 mg of Epinephrine in 10 mL of diluted acetic acid (1 in 500) and use this solution as the test solution. Take 1 mL of the test solution, add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is observed and it gradually changes to red.

(2) Place 1 mL each of the test solution obtained in (1) in test tubes, A and B. Add 10 mL of potassium biphthalate buffer solution, pH 3.5, in A and add 10 mL of phosphate buffer solution, pH 6.5, in B. To each of the test tubes, add 1 mL of iodine TS, allow to stand for 5 minutes and add 2 mL each of sodium thiosulfate TS: a red color is observed in test tube A and a deep red color is observed in test tube B.

Specific Optical Rotation $[\alpha]_D^{20}$: - 50.0 ~ - 53.5° (after drying, 1 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Epinephrine in 10 mL of dilute hydrochloric acid: the solution is clear and has no more color than Color Matching Fluid A.

(2) ***Adrenalone***—Dissolve 50 mg of Epinephrine in 0.05 mol/L hydrochloric acid TS to make exactly 25 mL and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry: it is not more than 0.40.

(3) ***Norepinephrine***—Dissolve 10.0 mg of Epinephrine in 2.0 mL of a L-tartaric acid solution (1 in 200). Pipet 1 mL of the solution, add 3.0 mL of pyridine, add 1.0 mL of freshly prepared sodium naphthoquinone sulfonate TS and allow to stand in a dark place for 30 minutes. Take this solution, add 5.0 mL of pyridine containing 50 mg of L-ascorbic acid: the solution has no more color than the following control solution.

Control solution—Dissolve 2.0 mg of Norepinephrine Tartrate RS and 90 mg of Epinephrine Tartrate RS in methanol to make exactly 10 mL. Pipet 1 mL of this solution and proceed in the same manner.

Loss on Drying Not more than 1.0 % (2 g, in vacuum, silica gel, 18 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Epinephrine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.321 mg of $C_9H_{13}NO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Epinephrine Injection

Epinephrine Hydrochloride Injection
Epirenamine Hydrochloride Injection
Adrenaline Hydrochloride Injection

Epinephrine Injection is an aqueous solution for injection. Epinephrine Injection contains not less than 0.085 w/v % and not more than 0.115 w/v % of epinephrine ($C_9H_{13}NO_3$: 183.20).

Method of Preparation Dissolve Epinephrine in diluted hydrochloric acid (9 in 10000) and prepare as directed under Injections.

Description Epinephrine Injection is a colorless, clear liquid.
Epinephrine Injection changes gradually to pale red and then to brown on exposure to air and light.

pH—2.3 ~ 5.0.

Identification (1) Take 1 mL of Epinephrine Injection, add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is observed and it gradually changes to red.

(2) Place 1 mL each of Epinephrine Injection in test tubes, A and B, and proceed as directed in the Identification (2) under Epinephrine.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 357.0 EU/mg of epinephrine.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

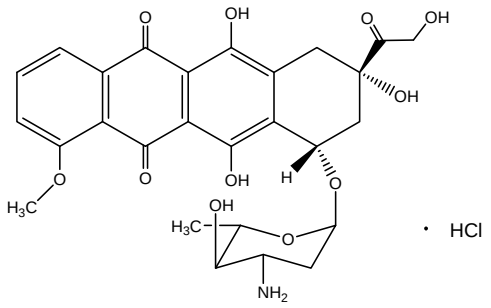
Assay Pipet 30 mL of Epinephrine Injection into a separatory funnel, add 25 mL of carbon tetrachloride, shake vigorously for 1 minute, allow the liquids to separate and discard the carbon tetrachloride layer. Repeat this procedure 3 times. Rinse the stopper and mouth of the separatory funnel, with a small amount of water. Add 0.2 mL of starch TS, then while shaking the separatory funnel, add iodine TS dropwise until a persistent blue color is observed and immediately add sodium thiosulfate TS to discharge the blue color. Add 2.1 g of sodium bicarbonate to the liquid in the separatory funnel, preventing it from coming in contact with the mouth of the separatory funnel and shake until most of the sodium bicarbonate dissolves. Rapidly inject 1.0 mL of acetic anhydride into the contents of the separatory funnel. Immediately stopper the separatory funnel loosely and allow to stand until the evolution of gas ceases. Shake vigorously, allow to stand for 5 minutes, extract with six 25 mL volumes of chloroform and filter each chloroform extract through a pledget of absorbent cotton. Evaporate the combined chloroform extracts in a water-bath in a current of air to 3 mL, completely transfer this residue by means of small portions of chloroform to a tared beaker and heat again to evaporate to dryness. Dry the residue at 105 ºC for 30 minutes, cool in a desiccator (silica gel) and accurately measure the weight, W (mg), of the dried residue. Dissolve in chloroform to make exactly 5 mL and determine the optical rotation, α_D, using a 100-mm cell as directed under the Optical Rotation Determination .

$$\text{Amount (mg) of epinephrine } (C_9H_{13}NO_3) = 0.5923 \times W \times \left(0.5 + \frac{0.5 \times \alpha_D}{93}\right)$$

Containers and Storage ***Containers***—Tight containers, and colored containers may be used.

Storage—Light-resistant.

Epirubicin Hydrochloride



$C_{27}H_{29}NO_{11}$·HCl: 579.98

(8*R*,10*S*)-10-((2*S*,4*S*,5*R*,6*S*)-4-Amino-5-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride
[*56390-09-1*]

Epirubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.
Epirubicin Hydrochloride contains not less than 970 μg (potency) and not more than 1020 μg (potency) of per mg of epirubicin hydrochloride ($C_{27}H_{29}NO_{11}$ · HCl), calculated on the anhydrous basis and corrected by the amount of the residual solvent.

Description Epirubicin Hydrochloride appears as pale yellowish red to brownish red powder.
Epirubicin Hydrochloride is soluble in water or in methanol, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.
Epirubicin Hydrochloride is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +310 ~ +340° (10 mg calculated on the anhydrous basis and corrected by the amount of the residual solvent, methanol, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 10 mg of Epirubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Absorbance $E_{1cm}^{1\%}$ (495 nm): 200 ~ 230 (15 mg calculated on the anhydrous basis and corrected by the amount of the residual solvent, methanol, 1000 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 50 mg of Epirubicin Hydrochloride in 5 mL of water: the solution is clear and dark red in color.

(2) ***Heavy metals***—Proceed with 1.0 g of Epirubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately an amount of Epirubicin Hydrochloride, equivalent to 50 mg (potency), dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the total area of the peaks other than epirubicin and 2-naphthalene-sulfonic acid by the area percentage method: not more than 5.0 %.

Internal standard solution—A solution of sodium 2-naphthalenesulfonate in a mixture of water, acetonitrile, methanol, and phosphoric acid (540 : 290 : 170 : 1) (1 in 2000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol, and phosphoric acid (540 : 290 : 170 : 1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epirubicin is about 9.5 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the system suitability solution (1). Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of epirubicin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the system suitability solution (1).

System performance: Dissolve 50 mg (potency) of Epirubicin Hydrochloride RS in the internal standard solution to make exactly 50 mL, and use this solution as the system suitability solution (2). When the procedure is run with 10 μL of this solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL each of the system suitability solution

(2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0 %.

Time span of measurement: About 3 times as long as the retention time of epirubicin beginning after the solvent peak

(5) ***Residual solvents***—Weigh accurately about 0.3 g of Epirubicin Hydrochloride, add exactly 0.6 mL of the internal standard solution, dissolve in *N,N*-dimethylformamide to make 6 mL, and use this solution as the test solution. Separately, pipet 1 mL of methanol, add *N,N*-dimethylformamide to make exactly 25 mL, and use this solution as the standard stock solution. Pipet 125 μL of acetone, 30 μL of ethanol (99.5), 32 μL of 1-propanol, and 17 μL of the standard stock solution, add exactly 10 mL of the internal standard solution, add *N,N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_{Ta}, Q_{Sa}, Q_{Tb}, Q_{Sb}, Q_{Tc}, Q_{Sc}, Q_{Td}, and Q_{Sd}, of the peak areas of acetone, ethanol, 1-propanol, and methanol to the peak area of the internal standard in each solution. Calculate the amounts of acetone, ethanol, 1-propanol, and methanol by the following equations: not more than 1.5 %, not more than 0.5 %, not more than 0.5 %, and not more than 0.1 %, respectively.

$$\text{Amount (\%) of acetone} = \frac{1}{W_T} \times \frac{Q_{Ta}}{Q_{Sa}} \times 593$$

$$\text{Amount (\%) of ethanol} = \frac{1}{W_T} \times \frac{Q_{Tb}}{Q_{Sb}} \times 142$$

$$\text{Amount (\%) of 1-propanol} = \frac{1}{W_T} \times \frac{Q_{Tc}}{Q_{Sc}} \times 154$$

$$\text{Amount (\%) of methanol} = \frac{1}{W_T} \times \frac{Q_{Td}}{Q_{Sd}} \times 2.23$$

W_T: Amount (mg) of Epirubicin Hydrochloride taken

Internal standard solution—A solution of 1,4-dioxane in *N,N*-dimethylformamide (1 in 100)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A fused silica column 0.53 mm in internal diameter and 30 m in length, the inside coated with polyethylene glycol for gas chromatography 1 μm in thickness.

Column temperature: Maintain at 40 °C for 11 minutes, and raise the temperature to 90 °C at the rate of 10 °C per minute. If necessary, raise the temperature to 130 °C at the rate of 50 °C per minute, and maintain at 130 °C for 30 minutes.

Injection port temperature: A constant temperature of about 120 °C

Detector temperature: A constant temperature of about 150 °C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 8 minutes.

Split ratio: 1 : 15

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone, methanol, ethanol, 1-propanol, and the internal standard are eluted in this order with the resolution between the peaks of acetone and the internal standard being not less than 30.

System repeatability: When the test is repeated 6 times with 1 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone, methanol, ethanol, and 1-propanol is not more than 4.0 %, respectively.

Water Not more than 8.0 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.5 % (0.1 g).

Sterility Test It meets the requirement, When Epirubicin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 1.1 EU/mg (potency) of epirubicin, when Epirubicin Hydrochloride is used in a sterile preparation.

Histamine It meets the requirement, when Epirubicin Hydrochloride is used in a sterile preparation. Weigh an appropriate amount of Epirubicin Hydrochloride, dissolve in water to make the solution so that each mL contains 2.0 mg (potency), and use the solution as the test solution. Use 0.5 mL of this solution for the test.

Assay Weigh accurately about 50 mg (potency) each of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS, add the internal standard solution to make exactly 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of epirubicin hydrochloride to that of the internal standard for the test solution and the standard solution.

Amount [μg (potency)] of epirubicin hydrochloride ($C_{27}H_{29}NO_{11}$·HCl)
= Amount [μg (potency)] of Epirubicin Hydrochloride RS $\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of sodium 2-naphthalenesulfonate in a mixture of water, acetonitrile, methanol, and phosphoric acid (540 : 290 : 170 : 1) (1 in 2000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 35 °C

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol, and phosphoric acid (540 : 290 : 170 : 1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epirubicin hydrochloride is about 9.5 minutes.

System suitability

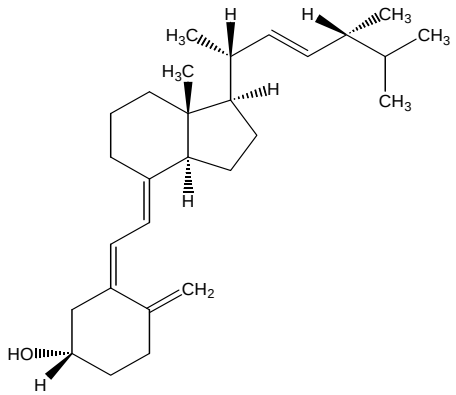
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—At a temperature between 0 °C and 5 °C.

Ergocalciferol



Calciferol

Vitamin D_2 $C_{28}H_{44}O$: 396.65

(5*Z*,7*E*,22*E*)-(3*S*)-9,10-Seco-5,7,10(19),22-ergostatetraen-3-ol [*50-14-6*]

Ergocalciferol contains not less than 97.0 % and not more than 103.0 % of ergocalciferol ($C_{28}H_{44}O$).

Description Ergocalciferol appears as white crystals, is odorless or has a faint, characteristic odor.
Ergocalciferol is freely soluble in ethanol (95), in chloroform, or in ether, sparingly soluble in isooctane and practically insoluble in water.
Ergocalciferol is affected by air and by light.

Melting point—115 ~ 118 ºC. Transfer Ergocalciferol to a capillary tube and dry for 3 hours in a desiccator (at a pressure not exceeding 2.67 kPa). Immediately fireseal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10 ºC below the expected melting point and heat at a rate of rise of about 3 ºC per minute and read the melting point.

Identification (1) Dissolve 0.5 mg of Ergocalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid and shake: a red color is observed and rapidly changes through purple and blue to green.

(2) Determine the infrared spectra of Ergocalciferol and Ergocalciferol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +102 ~ +107° (0.3 g, ethanol (95), 20 mL, 100 mm). Prepare the solution of Ergocalciferol within 30 minutes after the container has been opened and determine the rotation within 30 minutes after the solution has been prepared.

Absorbance $E_{1cm}^{1\%}$ (265 nm): 445 ~ 485 (10 mg, ethanol (95), 1000 mL).

Purity (1) ***Ergostrerol***—Dissolve 10 mg of Ergocalciferol in 2.0 mL of diluted ethanol (9 in 10), add allow 20 mg of digitonin in 2.0 mL of ethanol (9 in 10) and allow the mixture to stand for 18 hours: no precipitate is produced.

(2) ***Reducing substances***—To 10 mL of a solution of Ergocalciferol in ethanol (99.5) (1 in 100) add 0.5 mL of a solution of blue tetrazolium in methanol (1 in 200) and 0.5 mL of a solution of tetramethylammonium hydroxide in ethanol (99.5) (1 in 10), and allow to stand for 5 minutes. After exactly 5 minutes, add 1 mL of acetic acid (100), and use this solution as the test solution. Proceed with 10 mL of ethanol (99.5) in the same manner, and use this solution as the blank solution. Separately, proceed with a solution, prepared by dissolving a suitable amount of hydroquinone in anhydrous alcohol so that each mL

contains 0.2 μg, in the same manner, and use this solution as the standard solution. Determine the absorbances at 525 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank: the absorbance of the test solution is not more than that of the standard solution.

Assay Weigh accurately about 30 mg of Ergocalciferol and Ergocalciferol RS and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 to 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of ergocalciferol to that of the internal standard in each solution Perform the procedure rapidly avoiding contact with air or other oxidizing agents and using light-resistant containers.

$$\text{Amount (mg) of Ergocalciferol } (C_{28}H_{44}O) = \text{Amount (mg) of Ergocalciferol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dimethylphthalate in isooctane (1 in 100)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 10 cm to 30 cm in length, packed with a silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20 ºC.

Mobile phase: A mixture of hexane and *n*-amylalcohol (997 : 3).

Flow rate: Adjust the flow rate so that the retention time of Ergocalciferol is about 25 minutes.

System Suitability

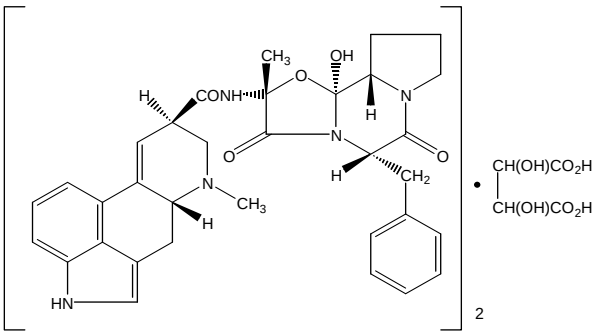
System performance: Dissolve 15 mg of Ergocalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil-bath under a reflux condenser for 2 hours and cool immediately to room temperature. Transfer the solution to a quartz test tube and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. Take 10 mL of this solution, add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution, as directed under the above operating conditions. the relative retention times of previtamin D_2, trans-vitamin D_2 and tachysterol$_2$ to that of ergoclaciferol are about 0.5, about 0.6 and about 1.1, respectively with resolution between previtamin D_2 and trans-vitamin D_2 being not less than 0.7 and that between ergocalciferol and tachysterol$_2$ being not less than 1.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ergocalciferol to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Ergotamine Tartrate



$(C_{33}H_{35}N_5O_5)_2{\cdot}C_4H_6O_6$: 1313.41

(5'*S*)-5'-Benzyl-12'-hydroxy-2'-methylergotaman-3',6',18-trione hemitartrate [*379-79-3*]

Ergotamine Tartrate contains not less than 98.0 % and not more than 101.0 % of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2{\cdot}C_4H_6O_6]$, calculated on the dried basis.

Description Ergotamine Tartrate appears as colorless crystals, or a white to pale yellowish white or grayish white, crystalline powder.

Ergotamine Tartrate is slightly soluble in water or in ethanol (95).

Melting point—About 180 ºC (with decomposition).

Identification (1) Dissolve 1 mg of Ergotamine Tartrate in 10 mL of a mixture of acetic acid (100) and ethyl acetate (1 : 1). Pipet 0.5 mL of this solution, add slowly 0.5 mL of sulfuric acid, with shaking in cold water and allow to stand: a purple color develops. Add 0.1 mL of diluted iron (III) chloride TS (1 in 12) to this solution: the color of the solution changes to blue or to blue-purple.

(2) Dissolve 1 mg of Ergotamine Tartrate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution, add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS and shake: a blue color develops.

Specific Optical Rotation Ergotamine base $[\alpha]_D^{20}$: -

155 ~ -165°. Dissolve 0.35 g of Ergotamine Tartrate in 25 mL of a solution of L-tartaric acid (1 in 100), add 0.5 g of sodium bicarbonate, shake gently and sufficiently and extract with four 10 mL volumes of ethanol-free chloroform. Filter the extracts successively through a small filter paper, moistened with ethanol-free chloroform, into a 50-mL volumetric flask. Allow the flask to stand in a water-bath at 20 ºC for 10 minutes and add ethanol-free chloroform (20 ºC) to make 50 mL and determine the optical rotation in a 100-mm cell. Separately, pipet 25 mL of this solution, evaporate to dryness under reduced pressure at a temperature not higher than 45 ºC, dissolve the residue in 25 mL of acetic acid (100) and titrate with 0.05 mol/L perchloric acid VS (indicator: 1 drop of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction. Calculate the specific optical rotation of the ergotamine base from the consumed volume of 0.05 mol/L perchloric acid VS and the optical rotation.

Each mL of 0.05 mol/L perchloric acid VS
= 29.084 mg of $C_{33}H_{35}N_5O_5$

Purity ***Related substances***—Perform this procedure without exposure to daylight, using light-resistant vessels. Weigh 40 mg of Ergotamine Tartrate, add exactly 10 mL of a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000), dissolve by thorough shaking and use this solution as the test solution. Pipet 1 mL of the test solution, add a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: any spot other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 5.0 % (0.1 g, in vacuum, 60 ºC, 4 hours).

Assay Weigh accurately about 0.2 g of Ergotamine Tartrate, dissolve in 15 mL of a mixture of acetic acid (100) and acetic anhydride (50 : 3) and titrate with 0.05 mol/L perchloric acid VS (indicator: 1 drop of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 32.836 mg of $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, almost well-filled or under nitrogen atmosphere, and not exceeding 5 ºC.

Ergotamine Tartrate Tablets

Ergotamine Tartrate Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$: 1313.41].

Method of Preparation Prepare as directed under Tablets, with Ergotamine Tartrate.

Identification Weigh accurately a portion of Ergotamine Tartrate Tablets, previously dried, equivalent to about 5 mg of labeled amount and make powder. Add 10 mL of hexane and shake and mix for a while. Place and delete hexane extracts and add 10 mL of chloroform saturated with ammonia solution (28) and shake and mix for a while, filter. Evaporate the filtrate in water bath. Dissolve the residue in 8 ml of a mixture of acetic acid (100) and ethyl acetate (1 : 1), pipet 1 mL of this solution. Shake and mix in ice bath and at the same time drop 1 mL of sulfuric acid: the solution is violet. Add 0.1 mL of diluted iron (III) chloride TS (1 in 2) in this solution: the color is blue and blue-purple.

Disintegration Test Disintegrate in 5 minutes for Tablets intended for sublingual use.

Dissolution Test This test is not applied to Sublingual Tablets. Take 1 tablet of Ergotamine Tartrate Tablet and perform the test at 75 revolutions per minute as directed in the Method 2 under Dissolution Test using 1000 mL of a solution of L-tartaric acid (1 in 100) as the dissolution solution. After 30 minutes from the start of the test, take the dissolved solution, filter and use the filtrate as the test solution. Separately, weigh accurately adequate amount of Ergotamine Tartrate RS, dissolve in dissolution solution to make a solution of known concentration and use this solution as the standard solution. Perform the test with the test solution and the standard solution at about 327 nm of the excitation and at about 427 nm of the fluorescence as directed under the Fluorometry, using dissolution solution as the control solution.
The dissolution rate (%) of Ergotamine Tartrate Tablets in 30 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement of the Content Uniformity Test when the test is performed with Ergotamine Tartrate Tablets according to the Assay.

Assay Weigh accurately and powder not less than 20 Ergotamine Tartrate Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$, add 50.0 mL of the internal standard and 300 mL of a mixture of acetonitrile and water (55 : 45) and sonicate for 10 minutes. Add a mixture of acetonitrile and water (55 :

45) to make exactly 500 mL, mix and filter. Discard the first 10 mL of the filtrate and use subsequent filtrate as the test solution. Separately weigh accurately about 10 mg of Ergotamine Tartrate RS, previously dried at 60 ºC for 4 hours in vacuum, dissolve in a mixture of acetonitrile and water (55 : 45) to make exactly 50 mL. Pipet 5 mL of this solution, add 5.0 mL of the internal standard solution and add a mixture of acetonitrile and water (55 : 45) to make exactly 50 mL. Use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak areas of Ergotamine Tartrate to that of the internal standard, respectively.

$$\text{Amount (mg) of ergotamine tartrate } (C_{33}H_{35}N_5O_5)_2{\cdot}C_4H_6O_6 = \text{Amount (mg) of Ergotamine Tartrate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 40 mg of Ergonobine Maleate in 250 mL of a mixture of acetonitrile and water (55 : 45).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 µm to10 µm in particle diameter).

Mobile phase: A mixture of acetonitrile: 0.01mol/L potassium dihydrogenphosphate (55 : 45).

Flow rate: 1 mL/minute.

System suitability

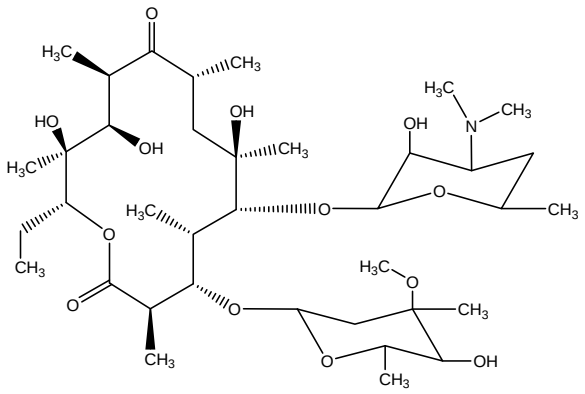
System performance: When the procedure is run with 20 µL of the standard solution, as directed under the above operating conditions, the symmetry factor of ergotamine is not more than 2.0 and the resolution between peaks of ergotamine and internal standard is not less than 3.0.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution under the above operating conditions, the relative standard deviation of ratios of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Erythromycin



$C_{37}H_{67}NO_{13}$: 733.93

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione [*114-07-8*]

Erythromycin is a macrolide substance having antibacterial activity produced by the growth of *Saccharopolyspora erythraea*.

Erythromycin contains not less than 930 µg (potency) and not more than 1020 µg (potency) per mg of erythromycin ($C_{37}H_{67}NO_{13}$), calculated on the anhydrous basis.

Description Erythromycin appears as white to pale yellowish white powder.

Erythromycin is very soluble in *N,N*-dimethylformamide, freely soluble in methanol or in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the infrared spectra of Erythromycin and Erythromycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg each of Erythromycin and Erythromycin RS in 1 mL of methanol, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 µL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (50 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 100 °C for 15 minutes: the spots obtained from the test solution and standard solution are deep purple in color and have the same R_f value.

Specific Optical Rotation $[\alpha]_D^{20}$: -71 ~ -78° (1 g calculated on the anhydrous basis, ethanol (95), 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Erythromycin in 150 mL of water is between 8.0 and 10.5.

Purity (1) ***Thiocyanate***—Weigh accurately about 0.1 g of Erythromycin, transfer to a 50 mL brown volumetric flask, dissolve in 20 mL of methanol, add 1 mL of iron (III) chloride TS and methanol to make 50 mL, and use this solution as the test solution. Separately, weigh accurately 0.1 g of potassium thiocyanate, previously dried at 105 °C for 1 hour and cooled, transfer to each of two 50 mL volumetric flasks, and add methanol to make 50 mL. Pipet 5.0 mL each of these solutions, add methanol to make 50 mL, pipet 5.0 mL each of these solutions, transfer to 50 mL brown volumetric flasks. To each of this solutions add exactly 1.0 mL of iron (III) chloride TS and methanol to make 50 mL, and use these solutions as the standard solutions. Separately, put 1 mL of iron (III) chloride in a 50 mL brown volumetric flask, dissolve in methanol to make 50 mL, and use this solution as the blank solution. Use the test solution, standard solutions, and blank solution within 30 minutes of the preparation of the solutions. Determine the absorbances at 492 nm of the test solution and standard solutions as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank, and calculate the amount of thiocyanate by the following equation: not more than 0.3 %.

$$\text{Content (\%) of thiocyanate} = \frac{58.08}{97.18} \times \frac{A_T}{W_T} \times 0.5 \times \left[\frac{W_1}{A_1} + \frac{W_2}{A_2}\right]$$

A_T: Absorbance of the test solution
W_T: Amount (mg) of Erythromycin taken
A_1, A_2: Absorbance of each standard solution
W_1, W_2: Amount (mg) of potassium thiocyanate taken for each standard solution
58.08: Molecular weight of thiocyanate
97.18: Molecular weight of potassium thiocyanate

System suitability: Determine the absorbance of each standard solution and calculate the suitability value (S) by the following equation: not less than 0.985 and not more than 1.015.

$$S = \frac{A_1}{W_1} \times \frac{W_2}{A_2}$$

A_1, A_2: Absorbance of each standard solution
W_1, W_2: Amount (mg) of potassium thiocyanate taken for each standard solution

(2) ***Heavy metals***—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Erythromycin according to Method 5, using hydrochloric acid instead of dilute hydrochloric acid (1 in 2), and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15 : 1) to make exactly 10 mL, and use this solution as the test solution. Separately, dissolve 16 mg of Erythromycin RS in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15 : 1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution and a mixture of phosphate buffer solution (pH 7.0) and methanol (15 : 1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate each peak area of each solution by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the test solution are not larger than those of erythromycin B and erythromycin C from the standard solution, and the each peak area other than erythromycin, erythromycin B, and erythromycin C is not larger than the peak area of erythromycin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 70 °C

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, ad adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 190 mL of *t*-butyl alcohol, 30 mL of acetonitrile, and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of erythromycin is about 20 minutes.

System suitability

System performance: Dissolve 2 mg of *N*-demethylerythromycin in 10 mL of the standard solution. When the procedure is run with 100 μL of this solution under the above operating conditions, *N*-demethyl-erythromycin, erythromycin C, erythromycin, and erythromycin B are eluted in this order with the resolutions between the peaks of *N*-demethylerythromycin and erythromycin C and between the peaks of *N*-demethyl-erythromycin and erythromycin being not less than 0.8 and not less than 5.5, respectively.

System repeatability: When the test is repeated 3 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of erythromycin is not more than 3.0 %.

Time span of measurement: About 4 times as long as the retention time of erythromycin beginning after the solvent peak

Water Not more than 10.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g).

Sterility Test It meets the requirement, when Erythromycin is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Erythromycin is used in a sterile preparation. Weigh appropriate amount of Erythromycin, dissolve in water, make the solution so that each mL contains 1.0 mg, and use the solution as the test solution. Use 5 mL of this solution for the test. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay Weigh accurately about 50 mg (potency) each of Erythromycin and Erythromycin RS, dissolve each in 25 mL of methanol, and add the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak area of erythromycin, A_T and A_S.

$$\text{Amount [μg (potency)] of erythromycin } (C_{37}H_{67}NO_{13}) = \text{Amount [μg (potency)] of Erythromycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 ~ 10 μm in particle diameter)

Mobile phase: The mixture of methanol and 0.067 mol/L of potassium dihydrogen phosphate (3:2).

System suitability

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of erythromycin is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Erythromycin Delayed-Release Capsules

Erythromycin Enteric-Coated Capsules

Erythromycin Delayed-Release Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Capsules, with Erythromycin.

Identification Proceed as directed in the Identification under Erythromycin Enteric-Coated Tablets.

Water Not more than 7.5 % (0.1 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Erythromycin Delayed-Release Capsules at 50 revolutions per minute for 60 minutes according to Method 1 under Dissolution Test, using 900 mL of 0.06 mol/L hydrochloric acid solution as the dissolution solution. Immediately wash the remaining contents and the sample container with water to remove the hydrochloric acid, then perform the test for 60 minutes under the same conditions as above, using 900 mL of 0.2 mol/L phosphate buffer solution (pH 6.8) as the dissolution solution. Take 5 mL of the dissolved solution 60 minutes after the start of the second test, filter, and use the filtrate as the test solution.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately the mass of the contents of not less than 20 Erythromycin Delayed-Release Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Enteric-Coated Tablets

Erythromycin Enteric-Coated Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Erythromycin Enteric-Coated Tablets are enteric-coated preparations.

Method of Preparation Prepare as directed under Tablets, with Erythromycin.

Identification Proceed as directed in the Identification (2) under Erythromycin. To an amount of powdered Erythromycin Enteric-Coated Tablets, equivalent to 10 mg (potency) according to the labeled potency, add 1 mL of methano, shake well, filter, and use the filtrate as the test solution.

Loss on Drying Not more than 10.0 % (0.2 g, 0.67 kPa, 60 °C, 3 hours)

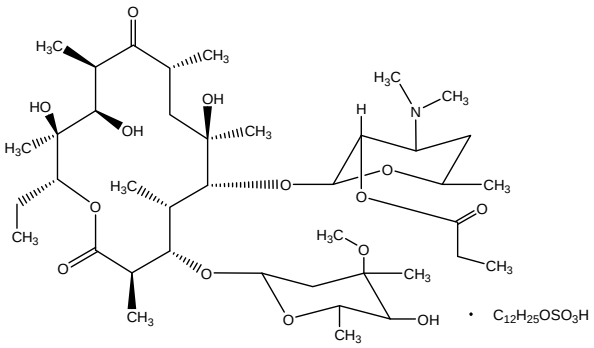
Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately and powder not less than 20 Erythromycin Enteric-Coated Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Estolate



$C_{40}H_{71}NO_{14}\cdot C_{12}H_{25}OSO_3H$: 1056.39

[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-2-[[(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-7,12,13-trihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-2,10-dioxo-oxacyclotetradec-6-yl]oxy]-6-methyloxan-3-yl] propanoate dodecyl hydrogen sulfate [*3521-62-8*]

Erythromycin Estolate contains not less than 600 μg (potency) per mg of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93), calculated on the anhydrous basis.

Description Erythromycin Estolate is a white crystalline powder or powder, is odorless, and has a bitter taste.

Erythromycin Estolate is sparingly soluble in methanol or in ethanol (95), and very slightly soluble in water or in benzene.

Identification Determine the infrared spectra of Erythromycin Estolate and Erythromycin Estolate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH The pH of a suspension obtained by suspending 0.1 g of Erythromycin Estolate in 10 mL of water is between 4.5 and 7.0.

Purity ***Free erythromycin***—Weigh accurately 0.250 g of Erythromycin Estolate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 75.0 mg of Erythromycin RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5.0 mL of this solution, add acetonitrile to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the peak area obtained from the test solution is not larger than area of the principal peak from the standard solution (not more than 6.0 %).

Buffer solution—Dissolve 3.4 g of potassium dihyrogen phosphate and 2.75 mL of triethylamine in water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 195 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: Adjust the pH of a mixture of acetonitrile and buffer solution (35 : 65) to 3.0 with dilute phosphoric acid.

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 25 μL of the standard solution and test solution under the above operating conditions, the retention times of erythromycin from the standard solution and the first principal peak of the test solution are about 5 minutes and about 10 minutes, respectively.

Time span of measurement: About 2 times as long as the retention time of the peak of erythromycin for the standard solution, about 4.5 times as long as the

retention time of the first peak of erythromycin propionate for the test solution.

Water Not more than 4.0 % (0.2 g, methanol containing 10 % imidazole, 20 mL, volumetric titration, direct titration).

Assay Perform the test according to Assay in Erythromycin. Weigh accurately an appropriate amount of of Erythromycin Estolate, dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solution so that each mL contains 1.0 mg (potency), and allow this solution to stand in 60 ºC water bath for 2 hours or at room temperature for 16 to 18 hours, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Estolate Capsules

Erythromycin Estolate Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Capsules, with Erythromycin Estolate.

Identification Dissolve a suitable amount each of Erythromycin Estolate Capsules and Erythromycin Estolate RS in a small amount of methanol, add water so that each mL contains 500 μg (potency), and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methylene chloride, methanol, benzene, and formamide (80 : 20 : 20 : 2 to 5) to a distance of about 10 cm, and air-dry the plate. Expose the plate to iodine vapor or spray evenly 10 % sulfuric acid solution on the plate: the spots obtained from the test solution and standard solution have the same R_f value.

Loss on Drying Not more than 5.0 % (0.1 g, in vacuum, 60 °C, 3 hours)

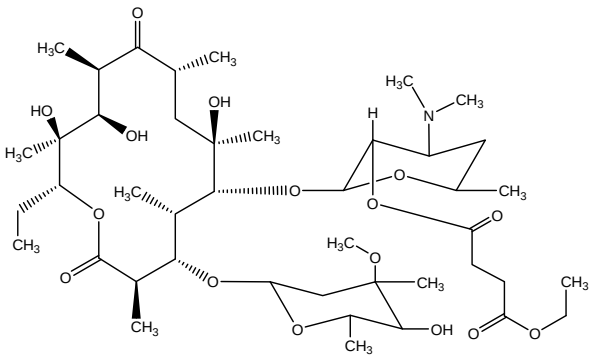
Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Asasy under Erythromycin. Weigh accurately the mass of the contents of not less than 20 Erythromycin Estolate Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1.0 mg (potency), then warm this solution in a water bath at 60 °C for 2 hours or allow to stand at room temperature for 16 to 18 hours, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Ethylsuccinate



$C_{43}H_{75}NO_{16}$: 862.05

4-O-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-2-[[(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-2,10-dioxo-oxacyclotetradec-6-yl]oxy]-6-methyloxan-3-yl] 1-O-ethyl-butanedioate [*1264-62-6*]

Erythromycin Ethylsuccinate is a derivative of erythromycin.
Erythromycin Ethylsuccinate contains not less than 780 μg (potency) and not more than 900 μg (potency) per mg of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93), calculated on the anhydrous basis.

Description Erythromycin Ethylsuccinate appears as white powder.
Erythromycin Ethylsuccinate is freely soluble in methanol or in acetone, soluble in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 3 mg of Erythromycin Ethylsuccinate in 2 mL of acetone, and add 2 mL of hydrochloric acid: the solution shows an orange color and immediately changes to red to deep purple.

(2) Determine the infrared spectra of Erythromycin Ethylsuccinate and Erythromycin Ethylsuccinate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH The pH of a suspension obtained by suspending

0.1 g of Erythromycin Ethylsuccinate in 10 mL of water is between 6.0 and 8.5.

Purity (1) ***Free erythromycin***—Weigh accurately 0.250 g of Erythromycin Ethylsuccinate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 75.0 mg of Erythroycin RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5.0 mL of this solution, add acetonitrile to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the peak area obtained from the test solution is not larger than the area of the principal peak from the standard solution (not more than 6.0 %).

Operating conditions

Proceed as directed in the operating conditions in the Purity – Free erythromycin under Erythromycin Estolate.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution and test solution under the above operating conditions, the retention times of the peaks of erythromycin from the standard solution and erythromycin ethylsuccinate from the test solution are about 8 minutes and about 24 minutes, respectively.

Time span of measurement: About 2 times as long as the retention time of the peak of erythromycin for the standard solution, about 2 times as long as the retention time of the peak of etrythromycin ethylsuccinate for the test solution

(2) ***Related substances***—Weigh accurately about 0.115 g of Erythromycin Ethylsuccinate, transfer to a 50 mL conical flask, dissolve in 25 mL of methanol, add 20 mL of the hydrolysis solution, mix, allow to stand at room temperature for about 12 hours, add the hydrolysis solution to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of erythromycin, dissolve in 12.5 mL of methanol, add the hydrolysis solution to make exactly 25 mL, and use this solution as the standard solution (1). Separately, weigh accurately 5 mg each of erythromycin B and erythromycin C, dissolve in 25 mL of methanol, add 2.5 mL of the standard solution (1) and the hydrolysis solution to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with 200 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography according to the following conditions, and determine the amount of each related substance other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin *N*-ethylsuccinate, and the amount of each of erythromycin A enol ether and erythromycin *N*-ethylsuccinate: not more than 3.0 %. The relative retention time of erythromycin *N*-ethylsuccinate with respect to the retention time of erythromycin A is about 1.3. Use the peak areas of erythromycin A enol ether and erythromycin *N*-ethylsuccinate obtained by the automatic integration method after multiplying by their response factors with respect to erythromycin A, 0.09 and 0.14, respectively.

$$\text{Amount (\%) of each related substance} = 50 \times \frac{C \times P}{W} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of erythromycin in the standard solution (2)

P: Content (%) of erythromycin A in Erythromycin RS

W: Amount (mg) of Erythromycin Ethylsuccinate taken

A_T: Peak area of each related substance other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin *N*-ethylsuccinate obtained from the test solution, and each of erythromycin A enol ether and erythromycin *N*-ethylsuccinate

A_S: Peak area of erythromycin A obtained from the standard solution (2)

Hydrolysis solution—Dissolve 2 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 8.0 with phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 70 °C

Mobile phae: To 50 mL of pH 8.0 buffer solution add 400 mL of water, 175 mL of *t*-butyl alcohol, 30 mL of acetonitrile, and water to make 1000 mL.

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with 200 μL of the system suitability solution (1) under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin A, and erythromycin B are eluted in this order with the resolutions between the peaks of *N*-demethylerythromycin and erythromycin C and between the peaks of *N*-demethylerythromycin and erythromycin A being not less than 0.8 and not less than 5.5, respectively. When the procedure is run with 100 μL of the system suitability solution (2) under the above operating conditions, the relative retention time of erythromycin A enol ether with respect to the retention time of erythromycin A is about 4.3 to 4.7.

System repeatability: When the test is repeated 5 times with 200 μL each of standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of erythromycin is not more than 1.0 %.

System suitability solution (1)—Dissolve about 2 mg of *N*-demethylerythromycin in 20 mL of the standard solution (2).

System suitability solution (2)—Dissolve about 10 mg of erythromycin in 2 mL of methanol, add 10 mL of pH 3.5 buffer solution, and allow to stand for 30 minutes. Keep this solution in a refrigerator and use within 8 hours.

pH 8.0 Buffer solution—Dissolve 2 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 8.0 with phosphoric acid.

pH 3.5 Buffer solution—Adjust the pH of 20 mL of pH 8.0 buffer solution to 3.5 with phosphoric acid.

Water Not more than 5.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 1.0 % (1 g).

Sterility Test It meets the requirement, when Erythromycin Ethylsuccinate is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Erythromycin Ethylsuccinate is used in a sterile preparation,. Weigh an appropriate amount of Erythromycin Ethylsuccinate, suspend in water to make the solution so that each mL contains 50 mg, and use the suspension as the test suspension. Inject 0.1 mL of this suspension into a muscle of a leg. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay ***The Cylinder-plate method method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (2) ② under Microbial Assay for Antibiotics. Adjust the pH of the solution so that it will be 7.8 to 8.0.

(2) Test organism- *Staphylococcus aureus* ATCC 6538 P.

(3) Weigh accurately about 50 mg (potency) of Erythromycin Ethylsuccinate, dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 50 mg of Erythromycin RS, dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5 °C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Ethylsuccinate Injection

Erythroycin Ethylsuccinate Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Injections, by dissolving Erythromycin Ethylsuccinate in polyethylene glycol 400.

Identification Weigh an amount of Erythromycin Ethylsuccinate Injection, equivalent to about 50 mg (potency), add methanol to make 25 mL, filter if necessary, and use the filtrate as the test solution. Separately, dissolve about 50 mg (potency) of Erythromycin Ethylsuccinate RS in methanol to make exactly 25 mL, filter if necessary, and use the filtrate as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), and water (3 : 1 : 1), and air-dry the plate. Expose the plate to iodine vapor or spray evenly 10 % sulfuric acid solution on the plate: the spots obtained from the test solution and standard solution have the same R_f value.

Water Not more than 1.5 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (2) ② under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be 7.8 to 8.0.

(2) Test organism- *Staphylococcus aureus* ATCC 6538P

(3) Pipet a suitable amount of Erythromycin Ethylsuccinate Injection, according to the labeled potency, add methanol so that each mL contains 1 mg (potency), pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer solution (pH 8.0), and use this solution as the test solution. Separately, weigh accurately about 25 mg (potency) of Erythromycin RS, dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, shake well, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Hermetic containers.

Erythromycin Gel

Erythromycin Gel contains not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

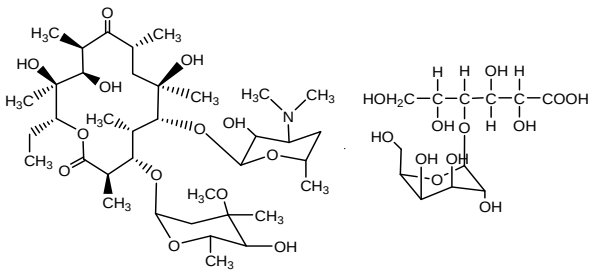
Method of Preparation Prepare as directed under Gels, with Erythromycin.

Identification Proceed as directed in the Identification (2) under Erythromycin Topical Solution. Weigh accurately a suitable amount each of Erythromycin Gel and Erythromycin RS, dissolve each in methanol so that each mL contains 2.5 mg (potency), and use these solutions as the test solution and standard solution, respectively.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately an amount of Erythromycin Gel, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Lactobionate



$C_{37}H_{67}NO_{13}\cdot C_{12}H_{22}O_{12}$: 1092.22

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione(2*R*,3*R*,4*R*,5*R*)-2,3,5,6-tetrahydroxy-4-[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyhexanoate [*3847-29-8*]

Erythromycin Lactobionate is the lactobionate of erythromycin.
Erythromycin Lactobionate contains not less than 590 μg (potency) and not more than 700 μg (potency) per mg of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93), calculated on the anhydrous basis.

Description Erythromycin Lactobionate is a white powder.
Erythromycin Lactobionate is freely soluble in water, in methanol, or in ethanol (99.5), and very slightly soluble in acetone.

Identification (1) To 3 mg of Erythromycin Lactobionate add 2 mL of acetone and 2 mL of hydrochloric acid: the solution shows an orange color and immediately changes to red to deep purple.

(2) To 0.3 g of Erythromycin Lactobionate add 15 mL of ammonia TS and 15 mL of chloroform, shake, and take the water layer. Wash this liquid with three 15 mL volumes of chloroform, and evaporate to dryness in a water bath. Dissolve the residue in 10 mL of a mixture of methanol and water (3 : 2), and use this solution as the test solution. Separately, dissolve 0.10 g of lactobionic acid in a mixture of methanol and water (3 : 2), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol, and acetic acid (100) (3 : 3 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105 °C for 20 minutes: the principal spot obtained from the test solution shows a deep brown color and the same R_f value as the principal spot from the standard solution.

pH The pH of a solution obtained by dissolving 0.5 g of Erythromycin Lactobionate in 10 mL of water is between 5.0 and 7.5.

Purity ***Heavy metals***—Proceed with 0.5 g of Erythromycin Lactobionate according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 50 ppm).

Water Not more than 5.0 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 2.0 % (1 g). Moisten the carbonized residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Sterility Test It meets the requirement, when Erythromycin Lactobionate is used in a sterile preparation.

Bacterial Endotoxins Less than 1.0 EU/mg (potency) of erythromycin, when Erythromycin Lactobionate is used in a sterile preparation.

Assay ***The Cylinder-plate method method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (2) ② under Microbial Assay for Antibiotics. Adjust the pH of the solution so that it will be 7.8 to 8.0.

(2) Test organism- *Staphylococcus aureus* ATCC 6538 P.

(3) Weigh accurately about 50 mg (potency) of Erythromycin Lactobionate, dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 50 mg of Erythromycin RS, dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5 °C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Lactobionate for Injection

Erythromycin Lactobionate for Injection is a preparation for injection, which is dissolved before use.
Erythromycin Lactobionate for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Injections, with Erythromycin Lactobionate.

Description Erythromycin Lactobionate for Injection appears as white powder.

Identification (1) To an amount of Erythromycin Lactobionate for Injection, equivalent to 5 mg of erythromycin lactobionate, add 2 mL of sulfuric acid and shake gently: a red-brown color is produced.

(2) Proceed as directed in the Identification (1) under Erythromycin Lactobionate.

pH The pH of a solution obtained by dissolving an amount of Erythromycin Lactobionate for Injection, equivalent to 0.5 g (potency) of erythromycin, in 10 mL of water is between 5.0 and 7.5.

Purity ***Heavy metals***—Proceed with 1.0 g of Erythromycin Lactobionate for Injection according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 5.0 mL of standard lead solution (not more than 50 ppm).

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Dissolve the contents of Erythromycin Lactobionate for Injection in water so that each mL contains 50 mg of erythromycin, and dilute so that each mL contains 0.2 mg of erythromycin. This solution is less than 0.07 EU/mL.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Erythromycin Lactobionate. Weigh accurately an amount of Erythromycin Lactobionate for Injection, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL. Pipet a suitable amount of

this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers.

Erythromycin Ophthalmic Ointment

Erythromycin Ophthalmic Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Ophthalmic Ointments, with Erythromycin.

Identification Proceed as directed in the Identification under Erythromycin Enteric-Coated Tablets. To a suitable amount of Erythromycin Ophthalmic Ointment add methanol, extract by warming in a water bath, filter to make a solution so that each mL contains 25 mg (potency), and use this solution as the test solution.

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration)

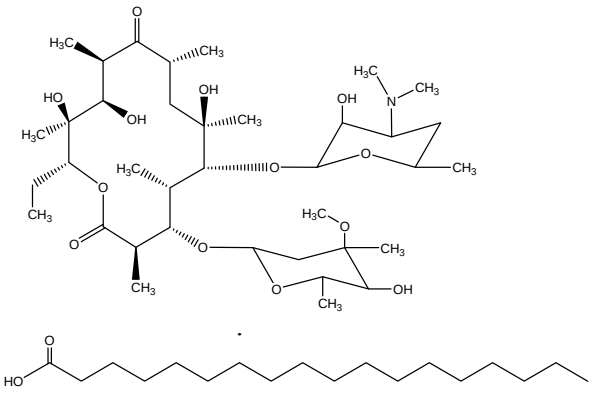
Sterility Test It meets the requirement.

Test for Metal Particles It meets the requirement.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately an amount of Erythromycin Ophthalmic Ointment, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Stearate



$C_{55}H_{103}NO_{15}$: 1018.40

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione octadecanoate [*643-22-1*]

Erythromycin Stearate is the stearate of erythromycin. Erythromycin Stearate contains not less than 600 μg (potency) and not more than 720 μg (potency) per mg of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93), calculated on the anhydrous basis.

Description Erythromycin Stearate appears as white powder.
Erythromycin Stearate is freely soluble in ethanol (95) or in acetone, soluble in methanol, and practically insoluble in water.

Identification (1) Dissolve 3 mg of Erythromycin Stearate in 2 mL of acetone, and add 2 mL of hydrochloric acid: the solution shows an orange color and immediately changes to red to deep purple.

(2) Determine the infrared spectra of Erythromycin Stearate and Erythomycin Stearate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH The pH of a solution obtained by dissolving 0.1 g of Erythromycin Stearate in 10 mL of water is between 6.0 and 11.0.

Purity ***Related substances***—Weigh accurately 0.165 g of Erythromycin Stearate, transfer to a 100 mL conical flask, dissolve in 15 mL of methanol, add 15 mL of pH 8.0 buffer solution, mix, filter through a filter with a pore size of 0.2 μm, and use the filtrate as the test solution. Separately, weigh accurately 6 mg each of

Erythromycin RS, erythromycin B, erythromycin C, and *N*-demethyl-erythromycin, dissolve in 15 mL of methanol, add 15 mL of pH 8.0 buffer solution, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the amount of each related substance other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and pseudoerythromycin A enol ether, and the amounts of erythromycin A enol ether and pseudoerythromycin A enol ether: not more than 3.0 %, respectively. The relative retention time of pseudoerythromycin A enol ether with respect to the retention time of erythromycin A is about 1.5. Use the peak areas of erythromycin A enol ether and pseudoerythromycin A enol ether obtained by the automatic integration method after multiplying by their response factors with respect to erythromycin A, 0.09 and 0.15, respectively.

$$\text{Amount (\%) of related substances} = 30 \times \frac{C \times P}{W} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of erythromycin in the standard solution

P: Content (%) of erythromycin A in Erythromycin RS

W: Amount (mg) of Erythromycin Stearate taken

A_T: Peak area of each related substance other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and pseudoerythromycin A enol ether obtained from the test solution, and each of erythromycin A enol ether and pseudoerythromycin A enol ether

A_S: Peak area of erythromycin A obtained from the standard solution

pH 8.0 Buffer solution—Dissolve 2 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 8.0 with phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene copolymer for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 70 °C

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 400 mL of water, 175 mL of *t*-butyl alcohol, 30 mL of acetonitrile, and water to make 1000 mL.

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin A, and erythromycin B are eluted in this order with the resolutions between the peaks of *N*-demethylerythromycin and erythromycin C and between *N*-demethylerythromycin and erythromycin A being not less than 0.8 and not less than 5.5, respectively. Separately, weigh accurately 5 mg of Erythromycin RS, dissolve in 1 mL of methanol, add 5 mL of pH 3.5 buffer solution, allow to stand for 30 minutes, and use this solution as the system suitability solution (1). When the procdure is run with 100 μL of this solution under the above operating conditions, the relative retention time of erythromycin A enol ether with respect to the retention time of erythromycin A is about 4.3 to 4.7.

pH 3.5 Buffer solution—Adjust the pH of 20 mL of pH 8.0 buffer solution to 3.5 with phosphoric acid.

System repeatability: Weigh accurately about 40 mg of Erythromycin RS, transfer to a 100 mL conical flask, dissolve in 5 mL of methanol, add 5 mL of pH 8.0 buffer solution, and use this solution as the system suitability solution (2). When the test is repeated 5 times with 100 μL of this solution under the above operating conditions, the relative standard deviation of the peak areas of erythromycin is not more than 2.0 %.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 2.0 % (1 g).

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately about 50 mg (potency) of Erythromycin Stearate, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Stearate Tablets

Erythromycin Stearate Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Tablets, with Erythromycin Stearate.

Identification To an amount of powdered Erythromycin Stearate Tablets, equivalent to about 100 mg (potency) according to the labeled potency, add 50 mL of chloroform, shake well, filter, and use the filtrate as the test solution. Separately, dissolve about 10 mg of

Erythromycin RS in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid, and water (3 : 1 : 1), and air-dry the plate. Expose the plate to iodine vapor or spray 10 % sulfuric acid solution: the spots obtained from the test solution and standard solution have the same R_f value.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration)

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately and powder not less than 20 Erythroycin Stearate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Erythromycin Topical Solution

Erythromycin Topical Solution is a topical solution.
Erythromycin Topical Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of Preparation Prepare as directed under Solutions, with Erythromycin.

Identification (1) To 1 mL of Erythromycin Topical Solution add acetone to make 10 mL, and add 2 mL of hydrochloric acid: the color of the solution changes through orange to reddish purple.

(2) To 1 mL of Erythromycin Topical Solution add 7 mL of methanol, and use this solution as the test solution. Separately, dissolve about 26 mg (potency) of Erythromycin RS in methanol to make 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and chloroform (85 : 15), and dry the plate at room temperature. Spray evenly a mixture of ethanol (95), *p*-methoxy-benzaldehyde, and sulfuric acid (90 : 5 : 5) on the plate, and heat at 100 °C for 10 minutes: the blackish purple spots from the test solution and standard solution have the same R_f value.

Water (1) If only ethanol is used as a solvent in the prescription: (i) Not more than 8.0 % when each mL contains 20 mg (potency). (ii) Not more than 5.0 % when each mL contains 15 mg (potency).

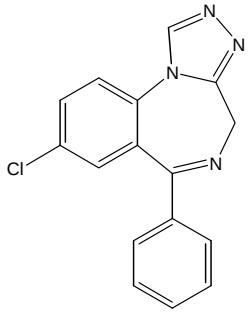
(2) If ethanol and acetone are used as solvents in the prescription: Not more than 2.0 %

(3) If ethanol as a solvent and a stabilizer are not used in the prescription, water requirement is not applicable.
Use a mixture of pyridine for water determination and methanol (1 : 1) instead of methanol for water determination.

Assay Proceed as directed in the Assay under Erythromycin. Weigh accurately an amount of Erythromycin Topical Solution, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of methanol, add the mobile phase to make exactly 50 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Estazolam



$C_{16}H_{11}ClN_4$: 294.74

8-Chloro-6-phenyl-4*H*-`1,2,4-triazolo[4,3-a]-1,4-benzodiazepine [*29975-16-4*]

Estazolam, when dried, contains not less than 98.5 % and not more than 101.0 % of estazolam ($C_{16}H_{11}ClN_4$).

Description Estazolam appears as white to pale yellowish white crystals or crystalline powder, is odorless and has a bitter taste.
Estazolam is soluble in methanol or in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in water or ether.

Identification (1) Dissolve 10 mg of Estazolam in 3 mL of sulfuric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine absorption spectra of solutions of

Estazolam and Estazolam RS, respectively, in 1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Estazolam as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 229 ~ 233 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Estazolam in 10 mL of ethanol (95): the solution is clear and colorless.

(2) ***Chloride***—Dissolve 1.0 g of Estazolam in 10 mL of ethanol by heating, add 40 mL of water, cool with shaking in ice-water, allow to stand to attain ordinary temperature and filter. To 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of ethanol (not more than 0.015 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Estazolam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***— Prepare the test solution with 1.0 g of Estazolam according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.2 g of Estazolam in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform and methanol (5 : 3 : 1) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution are not more intense than the principal spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 4 hours).

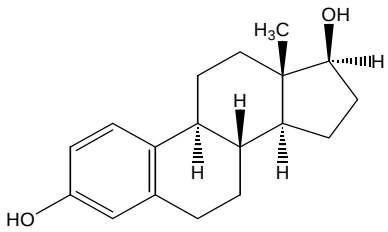
Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 0.25 g of Estazolam, previously dried, dissolve in 100 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, endpoint detection method in titrimetry), until the solution changes to the second equivalence point. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.737 mg of $C_{16}H_{11}ClN_4$

Containers and Storage ***Containers***—Well-closed containers.

Estradiol



$C_{18}H_{24}O_2$: 272.38

(17*β*)-Eestra-1,3,5(10)-triene-3,17-diol [*50-28-2*]

Estradiol, when dried, contains not less than 97.0 % and not more than 103.0 % of estradiol ($C_{18}H_{24}O_2$).

Description Estradiol appears as white crystals or crystalline powder and is odorless.
Estradiol is freely soluble in 1,4-dioxane, soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in sesame oil and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Estradiol and Estradiol RS, respectively, in ethanol (95) (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. The difference of absorption maxima in each absorption spectra is not less than 3.0 % at about 280 nm.

(2) Determine the infrared spectra of Estradiol and Estradiol RS as directed in the paste method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +76.0 ~ +83.0° (0.250 g calculated on the anhydrous basis, ethanol (95), 25 mL, 100 mm).

Melting Point 173 ~ 179 ºC.

Purity ***Related substances***—Weigh about 70 mg of Estradiol, add a mixture of *n*-butyl chloride and methanol (5 : 1), shake vigorously, add a mixture of *n*-butyl chloride and methanol (5 : 1) to make 10 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the peak area, A_i, of each related

substance and the total area of all peaks A_S by the area percentage method: the amount of each related substance is not more than 0.5 %, and the total amount of related substances is not more than 1.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and 25 cm in length, packed with porous silaznied silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of isooctane, *n*-butyl chloride, and methanol (45 : 4 : 1)

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the test solution under the above operating conditions, the resolution between the peaks of estradiol and each related substance is not less than 1.0, and the number of theoretical plates and symmetry factor are not less than 800 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 10 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak areas of estradiol is not more than 2.0 %.

Water Not more than 3.5 % (0.5 g, volume titration, direct titration).

Assay Weigh accurately about 0.1 g of Estradiol and dissolve in methanol to make exactly 250 mL. Pipet 10 mL of this solution, add 5.0 mL of the internal standard solution and 100 mL of methanol and water to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately a sufficient amount each of Estradiol RS (previously determined water contents as directed under water determination assay) and Estrone RS and dissolve separately in methanol to contain about 400 μg of Estradiol and 240 μg of Estrone in 1 mL. Pipet 10 mL each of these solutions, add 5.0 mL of the internal standard solution, 100 mL of methanol and add water to make exactly 200 mL and use these solutions as the standard solutions. Perform the test with 25 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of estradiol to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount(mg) of estradiol } (C_{18}H_{24}O_2) = 5 \times C \times \frac{Q_T}{Q_S}$$

C : Final concentration of Estradiol in the standard solution (μg/mL)

Internal standard solution—A solution of ethylparabene in methanol (0.6 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and water (55 : 45).

Flow rate: 1 mL/minute.

System suitability

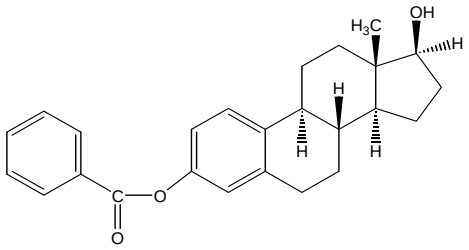
System performance: When the procedure is run with 25 μL of the standard solution, as directed under the above operating conditions, internal standard and estradiol and estrone are eluted in this order with the resolution between the peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution, as directed under the above operating conditions, the relative standard of the ratios of peak area of estradiol is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Estradiol Benzoate



$C_{25}H_{28}O_3$: 376.49

(17*β*)-3-Hydroxyestra-1,3,5(10)-trien-3-yl benzoate [*50-50-0*]

Estradiol Benzoate, when dried, contains not less than 97.0 % and not more than 101.0 % of estradiol benzoate ($C_{25}H_{28}O_3$).

Description Estradiol Benzoate is a white, crystalline powder and is odorless.

Estradiol Benzoate is sparingly soluble in acetone,

slightly soluble in methanol, in ethanol (95) or in ether, and practically insoluble in water.

Identification (1) Take 2 mg of Estradiol Benzoate, add 2 mL of sulfuric acid: a yellowish green color with a blue fluorescence is produced and the color of the solution changes to pale orange on the careful addition of 2 mL of water.

(2) Determine the infrared spectra of Estradiol Benzoate and Estradiol Benzoate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: + 54 ~ + 58° (after drying, 0.1 g, acetone, 10 mL, 100 mm).

Melting Point 191 ~ 198 ºC.

Purity (1) ***3,17α-Estradiol***—Dissolve 10.0 mg each of Estradiol Benzoate and Estradiol Benzoate RS in acetone to make exactly 200 mL and use these solutions as the test solution and the standard solution, respectively. Pipet 2 mL each of the test solution and the standard solution in separate glass-stoppered test tube, add boiling stones, evaporate the acetone by heating on a water-bath and dry the residue in a desiccator (in vacuum, P_2O_5) for 1 hour. Add 1.0 mL of dilute iron-phenol TS to each test tube. Stopper the test tubes loosely, heat for 30 seconds in a water-bath, shake in a water-bath for several seconds and heat for 2 minutes. Cool the solutions in ice for 2 minutes, add 4.0 mL of diluted sulfuric acid (7 in 20) and mix well: the solution obtained from the test solution has no more color than that from the standard solution.

(2) ***Related substances***—Dissolve 40 mg of Estradiol Benzoate in 2 mL of acetone and use this solution as the test solution. Pipet 1 mL of this solution, add acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of chloroform and diethylamine (19 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution are not more intense than the principal spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on Ignition Not more than 0.2 % (0.1 g).

Assay Weigh accurately about 10 mg each of Estradiol Benzoate and Estradiol Benzoate RS, previously dried and dissolve in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, add 5 mL of the internal standard solution, then add methanol to make 20 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of estradiol benzoate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of estradiol benzoate } (C_{25}H_{28}O_3) = \text{Amount (mg) of Estradiol Benzoate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of Progesterone in methanol (13 in 80000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 ºC.

Mobile phase: A mixture of acetonitrile and water (7 : 3).

Flow rate: Adjust the flow rate so that the retention time of Estradiol Benzoate is about 10 minutes.

System suitability

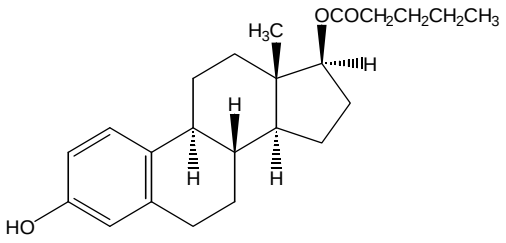
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and estradiol benzoate are eluted in this order with the resolutions between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviations of the ratios of the peak area of estradiol benzoate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Estradiol Valerate



$C_{23}H_{32}O_3$: 356.50

(17β)-3-Hydroxyestra-1,3,5(10)-trien-17-yl pentanoate [*979-32-8*]

Estradiol Valerate contains not less than 98.0 % and not more than 102.0 % of estradiol valerate ($C_{23}H_{32}O_3$).

Description Estradiol Valerate is a white crystalline powder, is odorless or has a slightly oily odor.
Estradiol Valerate is soluble in castor oil, in methanol, in benzylbenzoate or in 1,4-dioxane, sparingly soluble in sesame oil or peanut oil and practically insoluble in water.

Identification Determine the infrared spectra of Estradiol Valerate and Estradiol Valerate RS, prepared by adding chloroform and potassium bromide to Estradiol Valerate or Estradiol Valerate RS, then grinding and drying at 105 ºC, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +41 ~ +47° (0.500 g after drying, methanol, 20 mL, 100 mm).

Melting Point 143 ~ 150 ºC (Method 1).

Purity (1) ***Estradiol***—Dissolve 50 mg of Estradiol Valerate in 10 mL of acetone and use this solution as the test solution. Separately, dissolve 5 mg of Estradiol Valerate RS in 100 mL of acetone and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Then develop the plate with a mixture of cyclohexane and ethyl acetate (7 : 3) to a distance of about 15 cm and dry the plate at 90 ºC for 30 minutes. Spray evenly a solution of sulfuric acid in methanol (3 in 10) on the plate and heat at 90 ºC for 30 min: any spot other than the principal spot from the test solution or the spot corresponding to the Estradiol is not more intense or not larger than the spot from the standard solution (not more than 1.0 %).

(2) ***Free acid***—Neutralize 25 mL of ethanol (95), in a conical flask, with 0.01 mol/L sodium hydroxide VS to a pale blue color, using bromothymol blue TS. Accurately weigh about 0.50 g of Estradiol Valerate and dissolve Estradiol Valerate in the neutralized ethanol. Titrate rapidly with 0.01 mol/L sodium hydroxide VS to a pale blue color. (not more than 0.5 % of valeric aid)

Each mL of 0.01 mol/L sodium hydroxide VS
= 1.021 mg of $C_5H_{10}O_2$

(3) ***Related substances***—Dissolve about 0.1 g of Estradiol Valerate in 10 mL of acetone and use this solution as the test solution. Separately, weigh 10 mg of estradiol valerate RS and add acetone to make 10 mL. Pipet 0.1 mL, 0.5 mL, 1 mL and 2 mL of this solution and add acetone to each to make exactly 10 mL and use these solutions as the standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 20 μL each of the test solution and the standard solutions (1), (2), (3) and (4) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ether (4 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly sulfuric acid in ethanol (95) (1 in 10) on the plate, heat the plate until burn and cool. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm). Compare the intensities of any spot other than the principal spot from the test solution with those from the standard solution: the relative intensity is not more than 2.0 %.

Water Not more than 0.1 % (5 g, direct titration).

Assay Weigh accurately about 25 mg each of Estradiol Valerate and Estradiol Valerate RS, dissolve each in the internal standard solution to make exactly 25 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of estradiol valerate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of estradiol valerate}(C_{23}H_{32}O_3) = \text{Amount (mg) of Estradiol Valerate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Testosterone benzoate solution in tetrahydrofuran (2 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: Dissolve 0.8 g of ammonium nitrate in 300 mL of water, add 700 mL of acetonitrile and mix.

Flow rate: 2 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, estradiol valerate and the internal standard are eluted in this order with the resolution between their peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estradiol valerate to that of the internal standard is not more than

1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Estradiol Valerate Injection

Estradiol Valerate Injection is an oily solution for injection. Estradiol Valerate Injection contains not less than 90.0 % and not more than 115.0 % of the labeled amount of estradiol valerate ($C_{23}H_{32}O_3$: 356.50).

Method of Preparation Prepare as directed under Injections, with Estradiol Valerate.

Description Estradiol Valerate Injection appears as pale yellow oil liquid.

Identification Add 0.5 mL of Estradiol Valerate Injection in 10 mL of hexane and 10 mL of 80 % methanol in a separatory funnel and shake for 2 minutes. Allow to separate two layers. Add 1 mL of Folin's TS (dilute 1 mL with 2 mL of water before use) and 3 mL of sodium carbonate VS (1 in 5): a blue color is observed.

Purity ***Estradiol***—Weigh accurately a portion of Estradiol Valerate, add acetone to make a solution containing 30 % of estradiol according to the labeled amount. To 1.0 mL of this solution add oil labeled as vehicle to make exactly 10 mL and use this solution as the Estradiol solution. Perform the test with this solution and Estradiol Valerate Injection as directed under the Thin-layer chromatography. Spot 5 μL each of solutions on a plate coated with Thin-layer chromatography silica gel and proceed as directed in the Purity for Estradiol in Estradiol Valerate: not more than 3.0 %.

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Estradiol Valerate injection, equivalent to about 20 mg of estradiol valerate ($C_{23}H_{32}O_3$) according to the labeled amount. Rinse the pipet with tetrahydrofuran and add 5.0 mL of the internal standard solution, dilute with tetrahydrofuran to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Estradiol Valerate RS, add 5.0 mL of the internal standard solution and dilute with tetrahydrofuran to make exactly 25 mL and use this solution as the standard solution. Proceed as directed for the Assay under Estradiol Valerate.

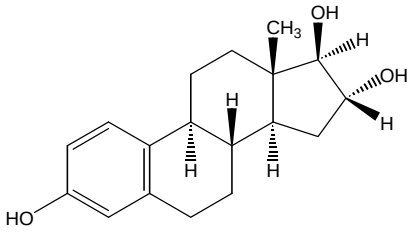
$$\text{Amount (mg) of estradiol valerate } (C_{23}H_{32}O_3) = \text{Amount (mg) of Estradiol Valerate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Testosterone benzoate solution in tetrahydrofuran (8 in 1000).

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Estriol



$C_{18}H_{24}O_3$: 288.38

(16*α*,17*β*)-Estra-1,3,5(10)-triene-3,16,17-triol [*50-27-1*]

Estriol, when dried, contains not less than 97.0 % and not more than 102.0 % of estriol ($C_{18}H_{24}O_3$).

Description Estriol is a white, crystalline powder and is odorless.

Estriol is sparingly soluble in methanol, slightly soluble in ethanol (95) or in 1,4-dioxane, and practically insoluble in water or in ether.

Identification (1) Dissolve 10 mg of Estriol in 100 mL of ethanol (95) by warming and use this solution as the test solution. Evaporate 1 mL of this solution on a water-bath to dryness, add 5 mL of a solution of sodium *p*-phenolsulfonate in phosphoric acid (1 in 50), heat at 150 ºC for 10 minutes and cool: a red-purple color develops.

(2) Dissolve 10 mg each of Estriol and Estriol RS separately in 100 mL ethanol (95) by warming and determine absorption spectra as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Estriol and Estriol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +60 ~ +65° (80 mg after drying, ethanol (99.5), 10 mL, 100 mm).

Melting Point 281 ~ 286 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Estriol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 40 mg of Estriol in 10 mL of ethanol (95) by warming and use this solution as the test solution. Pipet 1 mL of the test solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and acetic acid (100) (18 : 1 : 1 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate and heat at 105 ºC for 15 minutes: any spot other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, 105 ºC, 3 hours),

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 25 mg each of Estriol and Estriol RS, previously dried, and dissolve separately in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Estriol to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of Estriol } (C_{18}H_{24}O_3) = \text{Amount (mg) of Estriol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 1000).

Operating condition

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixed solution of water-methanol (51 : 49).

Flow rate: Adjust the flow rate so that the retention time of Estriol is about 10 minutes.

System suitability

System performance: When the test is performed with 10 μL of the standard solution under the above operating conditions, estriol and the internal standard are eluted in this order and the resolution between their peaks is not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviations of the peak area of estriol to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Estriol Tablets

Estriol Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of estriol ($C_{18}H_{24}O_3$: 288.38).

Method of Preparation Prepare as directed under Tablets, with Estriol.

Identification Weigh a portion of powdered Estriol Tablets, equivalent to 2 mg of Estriol according to the labeled amount, add 20 mL of ethanol (95), shake for 10 minutes, centrifuge and use the supernatant liquid as the test solution. Proceed with the test solution as directed in the Identification (1) and (2) under Estriol.

Dissolution Test Perform the test with 1 tablet of Estriol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water. Take 20 mL or more of the dissolved solution 30 minutes after starting the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add water to make exactly V' mL so that each mL contains about 0.1 μg of estriol ($C_{18}H_{24}O_3$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 10 mg of Estriol RS, previously dried at 105 ºC for 3 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and the standard solution according to the operating conditions as directed in the Assay under Estriol and determine the peak areas of Estriol, A_T and A_S, from the test solution and the standard solution, respectively.

The dissolution rate of Estriol Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of estriol } (C_{18}H_{24}O_3) = W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{9}{10}$$

W_S: Amount of Estriol RS (mg)
C: Labeled amount of estriol ($C_{18}H_{24}O_3$) in 1 tablet (mg)

Uniformity of Dosage Units It meets the requirement of the Content Uniformity Test when is performed the test according to the following method.
Take one tablet of Estriol Tablets, add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, add exactly 15 mL of methanol and shake for 15 minutes. Centrifuge this solution for 10 minutes, pipet a definite amount of the supernatant liquid and add methanol to make exactly a definite volume of solution so that each mL of the solution contains about 5 μg of estriol ($C_{18}H_{24}O_3$). Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution and use this solution as the test solution. Proceed with 20 μL of the test solution as directed in the Assay under Estriol. Use a solution of methyl benzoate in methanol (1 in 40000) as the internal standard solution. Calculate the mean value from each ratio of peak areas of 10 samples: the samples conform to the requirements of the deviation (%) of the mean value and each ratio of peak areas is within 15 %. If the deviation (%) exceeds 15 % and 1 sample shows deviation within 25 %, repeat the test with 20 samples. Calculate the deviation (%) of the mean value from each ratio of peak areas of the 30 samples used in the 2 tests and each ratio of peak areas: the samples conform to the requirements if the deviation exceeds 15 %, not more than 1 sample shows deviation within 25 %, and no sample shows deviation exceeding 25 %.

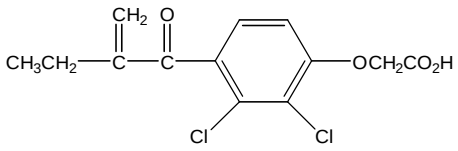
Assay Weigh accurately and powder not less than 20 Estriol Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of estriol ($C_{18}H_{24}O_3$), add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, shake with 25 mL of methanol for 10 minutes, centrifuge and take the supernatant liquid. Add 25 mL of methanol, repeat the above procedure twice, combine the supernatant liquid, add 5.0 mL of the internal standard solution, then add methanol to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Estriol RS, previously dried at 105 ºC for 3 hours and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add 5.0 mL of the internal standard solution, then add methanol to make 100 mL and use this solution as the standard solution. Proceed with 20 μL each of the test solution and the standard solution as directed in the Assay under Estriol.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = \text{Amount (mg) of Estriol RS} \times \frac{Q_T}{Q_S} \times \frac{1}{25}$$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 5000).

Containers and Storage ***Containers***—Tight containers.

Etacrynic Acid



$C_{13}H_{12}Cl_2O_4$: 303.14

[2,3-Dichloro-4-(2-methylenebutanoyl)phenoxy]acetic acid [*58-54-8*]

Etacrynic Acid, when dried, contains not less than 98.0 % and not more than 101.0 % of etacrynic acid ($C_{13}H_{12}Cl_2O_4$).

Description Etacrynic Acid is a white, crystalline powder, is odorless and has a slightly bitter taste.
Etacrynic Acid is very soluble in methanol, freely soluble in ethanol (95), in acetic acid (100) or in ether, and very slightly soluble in water.

Identification (1) Dissolve 0.2 g of Etacrynic Acid in 10 mL of acetic acid (100) and to 5 mL of this solution, add 0.1 mL of bromine TS: the color of the test solution disappears. To the remaining 5 mL of the solution, add 0.1 mL of potassium permanganate TS: the color of the test solution changes to pale orange immediately.

(2) Take 10 mg of Etacrynic Acid, add 1 mL of sodium hydroxide TS and heat in a water-bath for 3 minutes. After cooling, add 1 mL of chromotropic acid TS, and heat in a water-bath for 10 minutes: a deep purple color develops.

(3) Determine absorption spectra of solutions of Etacrynic Acid and Etacrynic Acid RS, respectively, in methanol (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Etacrynic Acid as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 121 ~ 125 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Etacrynic Acid in 10 mL of methanol: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Etacrynic

Acid according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prapare the test solution with 1.0 g of Etacrynic Acid according to Method 3 and perform the test. Add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30) and fire to burn (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Etacrynic Acid in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 3 mL of the test solution, add ethanol (95) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (6 : 5 : 2) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.25 % (1 g, in vacuum, 60 ºC, 2 hours).

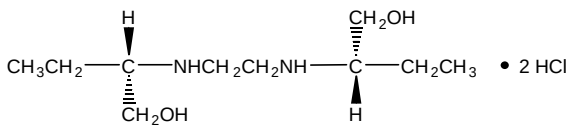
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of Etacrynic Acid, previously dried, place in an iodine bottle, dissolve in 20 mL of acetic acid (100) and add exactly 20 mL of 0.05 mol/L bromine VS. To this solution, add 3 mL of hydrochloric acid, stopper tightly at once, shake and allow to stand in a dark place for 60 minutes. Add carefully 50 mL of water and 15 mL of potassium iodide TS, stopper tightly at once, shake well and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L bromine VS
= 15.157 mg of $C_{13}H_{12}Cl_2O_4$

Containers and Storage ***Containers***—Well-closed containers.

Ethambutol Hydrochloride



$C_{10}H_{24}N_2O_2 \cdot 2HCl$: 277.23

(2*S*,2'*S*)-2,2'-(Ethane-1,2-diyldiimino)dibutan-1-ol dihydrochloride [*1070-11-7*]

Ethambutol Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

Description Ethambutol Hydrochloride appears as white crystals or crystalline powder, is odorless, and has a bitter taste.
Ethambutol Hydrochloride is very soluble in water, soluble in methanol or in ethanol (95), and practically insoluble in ether.
The pH of a solution of Ethambutol Hydrochloride (1 in 20) is between 3.4 and 4.0.

Identification (1) Take 10 mL of a solution of Ethambutol Hydrochloride (1 in 100), add 0.5 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a deep blue color is observed.

(2) Dissolve 0.1 g of Ethambutol Hydrochloride in 40 mL of water, add 20 mL of 2,4,6-trinitrophenol TS and allow to stand for 1 hour. Collect the precipitate, wash with 50 mL of water and dry at 105 ºC for 2 hours: the precipitate melts between 193 ºC and 197 ºC.

(3) A solution of Ethambutol Hydrochloride (1 in 30) responds to the Qualitative Tests for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: +5.5 ~ +6.1° (after drying, 5.0 g, water, 50 mL, 200 mm).

Melting Point 200 ~ 204 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ethambutol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Ethambutol Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***— Prapare the test solution with 1.0 g of Ethambutol Hydrochloride according to Method 1 and perform the test. (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Ethambutol Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Separately, dissolve 2 mg of Ethambutol Hydrochloride RS in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (18 : 1) to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of 0.5 % iodine in chloroform: the spot other than the principal spot obtained form the test solution is not more intense than the spot obtained from the standard solution (2.0 %).

(5) ***2-Aminobutanol***—Dissolve about 50 mg of Ethambutol Hydrochloride, weighed accurately, in water to make exactly 100 mL and use this solution as the test solution. Separately, dissolve adequate amount of

2-Amino-1-butanol RS, weighed accurately, in water and dilute sequentially to make a solution containing 5 μg per mL and use this solution as the standard solution. Transfer 10 mL of the test solution into 100 mL Erlenmeyer flask with glass stopper and add 10 mL of water and 20 mL of boric acid buffer solution. Separately, mix 10.0 mL of the test solution, 10.0 mL of the standard solution, and 20 mL of boric acid buffer solution in 100 mL Erlenmeyer flask with glass stopper. While the contents in two flasks are mixed on shaker, add quickly 10 mL of fluorescamine solution and stopper them and mix. Perform the test after exactly 1 minute with these solutions as directed under the Fluorimetry. Determine the fluorescence intensity at 385 nm of excitation wavelength and 485 nm of emission wavelength: the fluorescence intensity obtained from the test solution is not more than that of the difference between two solutions (not more than 1.0 %).

Boric acid buffer solution—Weigh about 1.24 g of boric acid, dissolve it in 90 mL of water, adjust with 5 mol/L sodium hydroxide to pH 9.0, and dilute with water to exactly 100 mL and mix.

Fluorescamine solution—Transfer 5 mg of fluorescamine into cylinder equipped with stopper and scale line, and dissolve in 50 mL of acetone.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Ethambutol Hydrochloride, previously dried, add 100 mL of acetic acid (100) and 5 mL of mercury (II) acetate TS for nonaqueous titration, mix with stirring, and titrate with 0.1 mol/L Perchloric acid VS until the color of the solution changes from blue to blue-green (indicator : methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.862 mg of $C_{10}H_{24}N_2O_2 \cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Ethambutol Hydrochloride Tablets

Ethambutol Hydrochloride Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$: 277.23).

Method of Preparation Prepare as directed under Tablets, with Ethambutol Hydrochloride.

Identification (1) Take a portion of powdered Ethambutol Hydrochloride Tablets, equivalent to 0.1 g of Ethambutol Hydrochloride according to the labeled amount, add 3 mL of methanol in a glass motor. Add 5 mL of methanol to make a suspension, then filter through a funnel lined with a filter paper previously moistened with methanol and collect the filtrate in a beaker containing 100 mL of acetone. Stir the mixture and allow crystallization to proceed for 15 minutes. Decant the clear supernatant liquid and gently dry the crystals with the aid of a current of air until the odor of methanol is no longer detectable. Determine the infrared spectra of this crystals and Ethambutol Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) An aqueous solution (1 in 10) of the crystals obtained in (1) responds to the Qualitative Tests for chloride.

Purity ***2-Aminobutanol***—Transfer adequate number of Ethambutol Hydrochloride Tablets, equivalent to 400 mg of Ethambutol Hydrochloride, into beaker, add acetone upto immersion of all sample, allow to stand for 15 minutes, decant acetone, dry. Powder the tablets removed the coating, add methanol, mix, dilute to exactly 100 mL and filter. Pipet 25 mL of the filtrate, dilute with water to make exactly 200 mL. After 15 minutes filter with dried filter paper, discard first turbid filtrate, use subsequent filtrate as the test solution. Then, proceed as directed in the Purity (4) under Ethambutol Hydrochloride.

Dissolution Test Perform the test with 1 tablet of Ethambutol Hydrochloride Tablets at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution 45 minutes after start of the test, filter, and use the filterate as the test solution. Separately, weigh accurately a portion of Ethambutol Hydrochloride RS, previously dried at 105 °C for 2 hours and dissolve in water to make 0.1 mg per mL and use this solution as the standard solution. Transfer 1 mL each of the test solution and the standard solution and water to 3 separate centrifuge test tubes with glass-stopper. Add 5.0 mL of bromocresol green solution and 10.0 mL of chloroform, stoppers and shake the mixture vigorously. Allow the mixture to separate, discard the upper aqueous layer and filter the 3 chloroform layers through separate pledges of cotton. Determine the absorption spectra with these solutions as directed under Ultraviolet-visible Spectrophotometry, at the wavelength of maximum absorbance at about 415 nm.
The dissolution rate of Ethambutol Hydrochloride Tablets in 45 minutes should be not less than 75 % (Q).

Phosphate buffer—Dissolve 38.0 g of sodium dihydrogen phosphate dihydrate and 2.0 g of anhy-

drous dibasic sodium phosphate in water to obtain 1000 mL of solution.

Bromocresol green solution—Dissolve 0.2 g of bromocresol green in 30 mL of water and 6.5 mL of 0.1 mol/L sodium hydroxide. Dilute with phosphate buffer to 500 mL, mix and add 0.1 mol/L hydrochloric acid to adjust to a pH of 4.6 ± 0.1.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh and finely powder not less than 20 Ethambutol Hydrochloride Tablets. Dissolve an accurately weighed amount of the powder, equivalent to about 30 mg of Ethambutol Hydrochloride according to the labeled amount, in water, shake to mix, add water to make exactly 100 mL and filter. Discard 10mL of first filtrate and use subsequent filtrate as the test solution. Separately, weigh accurately about 30 mg of Ethambutol Hydrochloride RS, dissolve in and dilte with water to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area, A_T and A_S, respectively.

$$\text{Amount (mg) of ethambutol hydrochloride } (C_{10}H_{24}N_2O_2\cdot 2HCl) = \text{Amount (mg) of Ethambutol Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Mobile phase: A mixture of the solution, prepared by mixing 1.0 mL of triethylamine with 1000 mL of water and adjusting with phosphoric acid to pH 7.0, and acetonitrile (1 : 1).

Flow rate: 1.0 mL/minute

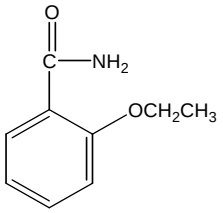
System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating condition, the symmetry factor of the peak of ethambutol are not more than 2.0.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethambutol is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Ethenzamide



Ethoxybenzamide $C_9H_{11}NO_2$: 165.19

2-Ethoxybenzamide [*938-73-8*]

Ethenzamide, when dried, contains not less than 98.0 % and not more than 101.0 % of ethenzamide ($C_9H_{11}NO_2$).

Description Ethenzamide appears as white crystals or crystalline powder.
Ethenzamide is soluble in methanol, in ethanol (95), or in acetone, and practically insoluble in water.
Ethenzamide begins to sublime slightly at about 105 °C.

Identification (1) Take 0.5 g of Ethenzamide, add 5 mL of sodium hydroxide TS and heat the mixture gently: the gas evolved turns moistened red litmus paper to blue.

(2) Take 0.2 g of Ethenzamide, add 10 mL of hydrobromic acid and boil the mixture gently for 1 hour under a reflux condenser. Cool in ice-water, filter and collect the separated crystalline precipitate, wash with three 5 mL volumes of ice-water and dry in a desiccator (in vacuum, silica gel) for 2 hours: the precipitate melts between 158 ºC and 161 ºC.

(3) Determine the absorption spectra of solutions of Ethenzamide and Ethenzamide RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Ethenzamide and Ethenzamide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 131 ~ 134 ºC.

Purity (1) ***Chloride***—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 6 mL of dilute nitric acid and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.7 mL of 0.01 mol/L hydrochloric acid VS, add 30 mL of acetone and 6 mL of dilute nitric acid and dilute with water to make 50 mL (not more than 0.050 %).

(2) ***Sulfate***—Dissolve 0.5 g of Ethenzamide in 30

mL of acetone, add 1 mL of dilute hydrochloric acid and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS, add 30 mL of acetone and 1 mL of dilute hydrochloric acid and dilute with water to 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Ethenzamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Take 0.40 g of Ethenzamide, add 0.3 g of potassium nitrate and 0.5 g of anhydrous sodium carbonate, mix thoroughly, ignite the mixture gradually and cool. Dissolve the residue in 10 mL of dilute sulfuric acid and heat the solution until white fumes begin to evolve. After cooling, add water carefully to make 5 mL, use this solution as the test solution and perform the test (not more than 5 ppm).

(5) ***Salicylamide***—Dissolve 0.20 g of Ethenzamide in 15 mL of dilute ethanol (2 in 3) and add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Loss on Drying Not more than 1.0 % (1 g, silica gel, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 20 mg each of Ethenzamide and Ethenzamide RS, previously dried and dissolve each in 70 mL of ethanol (95) by warming and after cooling, add ethanol (95) to make exactly 100 mL. Pipet 5.0 mL each of these solutions, add ethanol (95) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S of the test solution and the standard solution, respectively, at 290 nm as directed under Ultraviolet-visible Spectrophotometry, using ethanol (95) as the blank.

$$\text{Amount (mg) of ethenzamide } (C_9H_{11}NO_2) = \text{Amount (mg) of Ethenzamide RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Anesthetic Ether

$$CH_3CH_2OCH_2CH_3$$

$C_4H_{10}O$: 74.12

Ethoxyethane [*60-29-7*]

Anesthetic Ether contains not less than 96.0 % and not more than 98.0 % (by specific gravity) of ether ($C_4H_{10}O$). Anesthetic Ether contains small quantities of ethanol (95) and water. Suitable stabilizers may be added. Anesthetic Ether is not to be used for anesthesia if it has been removed from the original container for more than 24 hours.

Description Anesthetic Ether is a colorless, clear, mobile liquid, and has a characteristic odor.
Anesthetic Ether is miscible with ethanol (95).
Anesthetic Ether is soluble in water.
Anesthetic Ether is highly volatile and flammable.
Anesthetic Ether is slowly oxidized by the action of air and light, with the formation of peroxides.
The vapor of Anesthetic Ether, when mixed with air and ignited, may explode violently,

Boiling point—35 ~ 37 ºC.

Specific Gravity d_{20}^{20} : 0.718 ~ 0.721.

Purity (1) ***Characteristic odor***—Place 10 mL of Anesthetic Ether in an evaporating dish and allow to evaporate spontaneously to a volume of about 1 mL: no characteristic odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no characteristic odor is perceptible.

(2) ***Acid***—Place 10 mL of diluted ethanol (4 in 5) and 0.5 mL of phenolphthalein TS in a glass-stoppered flask and add 0.02 mol/L sodium hydroxide VS dropwise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Anesthetic Ether, stopper the flask, shake gently and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) ***Aldehyde***—To 100 mL of water in a glass-stoppered flask add 10 mL of Anesthetic Ether and 1 mL of a solution of sodium bisulfite (1 in 1000), stopper tightly, shake vigorously for 10 seconds and allow the mixture to stand in a cool place for 30 minutes, protected from light. Add 2 mL of starch TS and add dropwise 0.01 mol/L iodine VS until a pale blue color develops. Shake with about 2 g of sodium bicarbonate to decolorize the solution and add 1 mL of diluted 0.01 mol/L iodine VS (9 in 40): a blue color develops. Keep the temperature of the solution below 18 ºC during the procedure.

(4) ***Peroxide***—Place 10 mL of Anesthetic Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake occasionally for 1 hour, protecting from light, then add 1 mL of starch TS and shake well: no color is produced in the aqueous layer and in the ether layer.

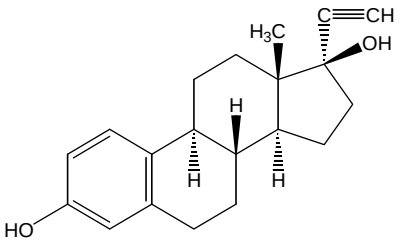
(5) ***Residue on evaporation***—Evaporate 50 mL of Anesthetic Ether and dry the residue at 105 ºC for 1 hour: the weight of the residue is not more than 1.0 mg.

Water Not more than 0.2 % (0.1 g, volumetric titration, direct titration).

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, without fill up, remote from fire, and not exceeding 25 ºC.

Ethinylestradiol



$C_{20}H_{24}O_2$: 296.40

19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol
[*57-63-6*]

Ethinylestradiol, when dried, contains not less than 98.0 % and not more than 101.0 % of ethinyestradiol ($C_{20}H_{24}O_2$).

Description Ethinylestradiol appears as white to pale yellow crystals or crystalline powder and is odorless.
Ethinylestradiol is freely soluble in pyridine or in tetrahydrofuran, soluble in ethanol (95) or in ether, and practically insoluble in water.
Ethinylestradiol dissolves in sodium hydroxide TS.

Identification (1) Dissolve 2 mg of Ethinylestradiol in 1 mL of a mixture of ethanol (95) and sulfuric acid (1 : 1): a purplish red color develops with a yellow-green fluorescence. Add carefully 2 mL of water to this solution: the color of the solution changes to red-purple.

(2) Transfer 20 mg of Ethinylestradiol to a test tube with glass-stopper, dissolve in 10 mL of a solution of potassium hydroxide (1 in 20), add 0.1 g of benzoyl chloride and shake. Collect the resulting precipitate, recrystallize from methanol and dry in a desiccator (in vacuum, P_2O_5): the precipitate melts between 200 ºC and 202 ºC.

Specific Optical Rotation $[\alpha]_D^{20}$: -26 ~ -31° (after drying, 0.1 g, pyridine, 25 mL, 200 mm).

Melting Point 180 ~ 186 ºC or 142 ~ 146 ºC.

Purity ***Estrone***—Dissolve 5 mg of Ethinylestradiol in 0.5 mL of ethanol (95) and add 50 mg of *m*-dinitrobenzene. Add 0.5 mL of freshly prepared dilute potassium hydroxide-ethanol TS, allow to stand in a dark place for 1 hour and add 10 mL of ethanol (95): the solution has no more color than the following control solution.

Control solution—Proceed in the same manner as mentioned above, omitting Ethinylestradiol.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.2 g of Ethinylestradiol, previously dried, and dissolve in 40 mL of tetrahydrofurane. Add 10 mL of a solution of silver nitrate (1 in 20) and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.640 mg of $C_{20}H_{24}O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ethinylestradiol Tablets

Ethinylestradiol Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of Preparation Prepare as directed under Tablets, with Ethinylestradiol.

Identification (1) Evaporate to dryness 5 mL of the test solution obtained in Assay and add 2 mL of a mixture of sulfuric acid and ethanol (2 : 1) to the residue: a pale red color with a yellow fluorescence develops. To the solution, add carefully 4 mL of water: the color of the solution changes to red-purple.

(2) Evaporate to dryness 10 mL of the test solution obtained in Assay, add 0.2 mL of acetic acid (31) and 2 mL of phosphoric acid to the residue and heat in a water-bath for 5 minutes: a red color with a yellow-green fluorescence develops.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement of the Content Uniformity Test when the test is performed according to the following method. Place 1 tablet of Ethinylestradiol Tablets in a separator, add 10 mL of the 2nd fluid of test fluids under the Disintegration Test and shake until the tablet is disintegrated. Add 10 mL of dilute sulfuric acid and 20 mL of chloroform, shake vigorously for 5 minutes and filter the chloroform layer into a conical flask through filter paper on which 5 g of anhydrous sodium sulfate is placed. Extract the aqueous layer with two 20 mL volumes of chloroform, proceed with the extracts in the same manner as before and combine the filtrates with the previous one. Evaporate gently the combined filtrate in a water-bath with the aid of a current of nitrogen, dis-

solve the residue in exactly 100 mL of methanol and centrifuge, if necessary. Pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about 0.04 μg of ethinylestradiol ($C_{20}H_{24}O_2$) per mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours, dissolve in methanol, dilute to a volume containing about 0.04 μg of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the standard solution. Pipet 4 mL of sulfuric acid-methanol TS into three test tubes with glass-stopper, T, S and B, cool in ice, to each tube, add exactly 1 mL each of the test solution, the standard solution and methanol, shake immediately and allow to stand in a water-bath at 30 ºC for 40 minutes. And allow to stand in a water-bath at 20 ºC for 5 minutes. Perform the test with the test solution and the standard solution as directed under the Fluorometry. Determine the fluorescence intensities, F_T, F_S and F_B, of the test solution, the standard solution and the blank solution, respectively, using the fluorophotometer at about 460 nm of the excitation and at about 493 nm of the fluorescence.

$$\text{Amount (mg) of ethinylestradiol } (C_{20}H_{24}O_2) = \text{Amount (mg) of Ethinylestradiol RS} \times \frac{F_T - F_B}{F_S - F_B} \times \frac{V}{2500} \times \frac{1}{x}$$

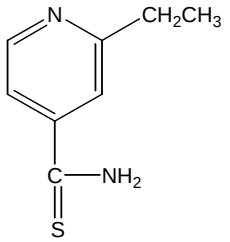
Assay Weigh accurately and powder not less than 20 Ethinylestradiol Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of ethinylestradiol ($C_{20}H_{24}O_2$), place in a 50 mL beaker, add 2 mL of water, shake well, add 3 mL of chloroform and shake well again. Add 4 g of siliceous earth for chromatography, mix well until the contents do not stick to the inner wall of the beaker and use the substance as the sample. Take the chromatographic column, add the sample with a funnel and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography and place in the chromatographic tube. Wipe off the test solution sticking to the beaker and the tamping rod with glass wool and place in the chromatographic tube. Push down the sample and press lightly on the chromatographic column to make the height of the column, 110 mm to 130 mm. Take 70 mL of chloroform, rinse the inner wall of the chromatographic tube with a portion of the chloroform and transfer the remaining portion to the chromatographic tube. Collect the efficient solution at a flow rate not more than 0.8 mL per minute. After completing the elution, rinse the lower end of the chromatographic tube with a small quantity of chloroform, add chloroform to make exactly 100 mL and use this solution as the test solution. Weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution and add chloroform to make exactly 100 mL. Use this solution as the standard solution. Transfer exactly 6 mL each of the test solution and the standard solution to each separators and add 20 mL of isooctane. Add exactly 10 mL of a mixture of sulfuric acid and methanol (7 : 3), shake vigorously for 5 minutes, allow to stand in a dark place for 15 minutes and centrifuge. Perform the test with the resulting color solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution, prepared with 6 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions obtained from the test solution and the standard solution at 540 nm, respectively.

Chromatographic column: Pack a pledget of glass wool in the bottom of a column 25 mm in internal diameter and about 30 cm in length and place 5 g of anhydrous sodium sulfate on the glass wool. Separately, place 5 g of siliceous earth for chromatography in a 200-mL beaker, soak well in 4 mL of 1 mol/L hydrochloric acid TS and mix uniformly. Put the siliceous earth into the chromatographic column in small portions to make 60 to 80 mm in length in proper hardness with 1 tamping rod.

$$\text{Amount (mg) of ethinylestradiol } (C_{20}H_{24}O_2) = \text{Amount (mg) of Ethinylestradiol RS} \times \frac{A_T}{A_S} \times \frac{1}{20}$$

Containers and Storage ***Containers***—Tight containers.

Ethionamide



$C_8H_{10}N_2S$: 166.24

2-Ethylpyridine-4-carbothioamide [*536-33-4*]

Ethionamide, when dried, contains not less than 98.5 % and not more than 101.0 % of ethionamide ($C_8H_{10}N_2S$).

Description Ethionamide appears as yellow crystals or crystalline powder, and has a characteristic odor and taste.

Ethionamide is soluble in methanol or acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in ether, and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Ethionamide and Ethionamide RS, respectively, in methanol (3 in 160000) as directed under Ultraviolet-visible Spectrophotometry: both spectra

exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ethionamide and Ethionamide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 161 ~ 165 ºC.

Purity (1) ***Acid***—Dissolve 3.0 g of Ethionamide in 30 mL of methanol by warming, add 90 mL of water, allow to stand in ice-water for 1 hour and filter. Take 80 mL of the filtrate, add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) ***Heavy metals***—Proceed with 1.0 g of Ethionamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Ethionamide and perform according to Method 3. Add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30) and fire to burn (not more than 2 ppm).

(4) ***Selenium***—Proceed with 0.2 g of Ethionamide as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1000 mL flask as a combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 with diluted ammonia solution (28) (1 in 2), add water to make 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(5) ***Related substances***—Proceed this procedure without exposure to light, using light-resistant vessels. Dissolve 0.20 g of Ethionamide in 10 mL of methanol and use this solution as the test solution. Pipet 0.5 mL of test solution, add acetone to make exactly 100 mL and use this solution as the standard solution (1). Separately, pipet 0.2 mL of the test solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and methanol (6 : 2 : 1) to a distance of about 15 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot obtained with the test solution is not more intense than the corresponding spot with the standard solution (1), and the number of the spots other than the principal spot obtained with the test solution being more intense than that with the standard solution (2) is not more than one.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

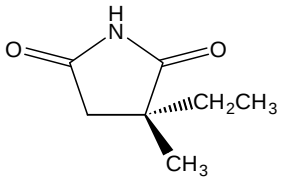
Assay Weigh accurately about 0.3 g of Ethionamide, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of 1-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.624 mg of $C_8H_{10}N_2S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Ethosuximide



and enantiomer

$C_7H_{11}NO_2$: 141.17

3-Ethyl-3-methylpyrrolidine-2,5-dione [*77-67-8*]

Ethosuximide contains not less than 98.5 % and not more than 101.0 % of ethosuximide ($C_7H_{11}NO_2$) calculated on the anhydrous basis.

Description Ethosuximide is a white, paraffin-like

solid or powder, is odorless or has a slight, characteristic odor.

Ethosuximide is very soluble in methanol, in ethanol (95), in ether, or in *N,N*-dimethylformamide, and freely soluble in water.

Melting point—About 48 ºC.

Identification (1) Take 0.2 g of Ethosuximide, add 10 mL of sodium hydroxide TS and boil: the gas evolved turns a moistened red litmus paper blue.

(2) Dissolve 50 mg of Ethosuximide in 1 mL of ethanol (95), add 3 drops of a solution of copper (II) acetate (1 in 100), warm slightly and add 1 to 2 drops of sodium hydroxide TS: a purple color is produced.

(3) Determine the absorption spectra of the solutions of Ethosuximide and Ethosuximide RS, respectively, in ethanol (95) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ethosuximide in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—With 1.0 g of Ethosuximide, perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Ethosuximide according to Method 1. and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prapare the test solution with 1.0 g of Ethosuximide and perform the test according to Method 1(not more than 2 ppm).

(5) ***Acid anhydride***—Dissolve 0.50 g of Ethosuximide in 1 mL of ethanol (95), add 1 mL of hydroxylamine hydrochloride-iron (III) chloride TS and allow to stand for 5 minutes. Add 3 mL of water, mix and allow to stand for 5 minutes: the red to red-purple color of this solution is not more intense than that of the following control solution.

Control solution—Dissolve 70 mg of succinic anhydride in ethanol (95) to make exactly 100 mL. To 1.0 mL of this solution, add 1 mL of hydroxylamine hydrochloride-iron (III) chloride TS and proceed in the same manner.

(6) ***Cyanide***—Dissolve 1.0 g of Ethosuximide in 10 mL of ethanol (95) and add 3 drops of iron (II) sulfate TS, 1 mL of sodium hydroxide TS and 2 to 3 drops of iron (III) chloride TS. Warm gently and acidify with dilute sulfuric acid: not a blue precipitate and a blue color are produced within 15 minutes.

(7) ***Related substances***—Weigh accurately 1.0 g of Ethosuximide, dissolve in the mobile phase to make 10 mL, and use this solution as the test solution. Separately, weigh accurately 10.0 mg each of Ethosuximide RS and 2-ethyl-2-methylsuccinic acid, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the amount of 2-ethyl-2-methylsuccinic acid by equation 91): not more than 0.1 %. Calculate the amount of each related substance by equation (2): not more than 0.1 %, and the total amount of related substances is not more than 0.5 %.

$$\text{Amount (\%) of 2-ethyl-2-methylsuccinic acid} = \frac{C}{W} \times \frac{A_T}{A_S} \quad (1)$$

C: Concentration (mg/mL) of 2-ethyl-2-methylsuccinic acid in the standard solution

W: Amount (g) of Ethosuximide taken

A_T: Peak area of 2-ethyl-2-methylsuccinic acid obtained from the test solution

A_S: Peak area of 2-ethyl-2-methylsuccinic acid obtained from the standard solution

$$\text{Amount (\%) of related substances} = \frac{C}{W} \times \frac{A_i}{A_S} \quad (2)$$

C: Concentration (mg/mL) of ethosuximide in the standard solution

W: Amount (g) of Ethosuximide taken

A_i: Peak area of each related substance obtained from the test solution

A_S: Peak area of ethosuximide obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadeylsilanized silica gel for liquid chromatography.

Mobile phase: A mixture of pH 3.0 phosphate buffer solution and acetonitrile (9 : 1)

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately a suitable amount each of 2-ethyl-2-methylsuccinic acid and Ethosuximide RS, dissolve in the mobile phase so that each mL contains 2 mg of 2-ethyl-2-methylsuccinic acid and 10 mg of ethosuximide, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of 2-ethyl-2-methylsuccinic acid and ethosuximide is not less than 6.6, the number of theoretical plates is not less than 2900, and the symmetry factor of the peak of ethosuximide is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution

under the above operating conditions, the relative standard deviation of the peak areas of ethosuximide and 2-ethyl-2-methylsuccinic acid is not more than 0.4 %, respectively.

Phosphate buffer solution, pH 3.0—To 4.1 mL of phosphoric acid add water to make 1000 mL, and adjust the pH to 3.0 with sodium hydroxide.

Water Not more than 0.5 % (2 g, volumetric titration,direct titration).

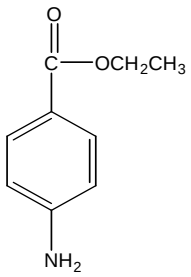
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Ethosuximide, dissolve in 20 mL of *N,N*-dimethyl-formamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary connection.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 14.117 mg of $C_7H_{11}NO_2$

Containers and Storage ***Containers***—Tight containers.

Ethyl Aminobenzoate



Benzocaine
Anesthezine $C_9H_{11}NO_2$: 165.19

Ethyl 4-aminobenzoate [*94-09-7*]

Ethyl Aminobenzoate, when dried, contains not less than 99.0 % and not more than 101.0 % of ethyl aminobenzoate ($C_9H_{11}NO_2$).

Description Ethyl Aminobenzoate appears as white crystals or crystalline powder and is odorless.
Ethyl Aminobenzoate has a slightly bitter taste, numbing the tongue.
Ethyl Aminobenzoate is freely soluble in ethanol (95) or in ether, and very slightly soluble in water.
Ethyl Aminobenzoate dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 10 mg of Ethyl Aminobenzoate in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 0.1 g of Ethyl Aminobenzoate in 5 mL of water with the aid of dilute hydrochloric acid added drop-wise and add iodine TS drop-wise: a brown precipitate is produced.

(3) Warm 50 mg of Ethyl Aminobenzoate with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

Melting Point 89 ~ 91 ºC.

Purity (1) ***Acid***—Dissolve 1.0 g of Ethyl Aminobenzoate in 10 mL of neutralized ethanol and add 10 mL of water, 2 drops of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(2) ***Chloride***—Dissolve 0.20 g of Ethyl Aminobenzoate in 5 mL of ethanol (95), add 2 to 3 drops each of dilute nitric acid and of silver nitrate TS: no change occurs immediately.

(3) ***Heavy metals***—Dissolve 2.0 g of Ethyl Aminobenzoate in 20 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and sufficient ethanol (95) to make 50 mL (not more than 10 ppm).

(4) ***Readily carbonizable substances***—Perform the test with 0.5 g of Ethyl Aminobenzoate: the solution has no more color than Matching Fluid A.

Loss on Drying Not more than 1.0 % (1 g, silica gel, 3 hours).

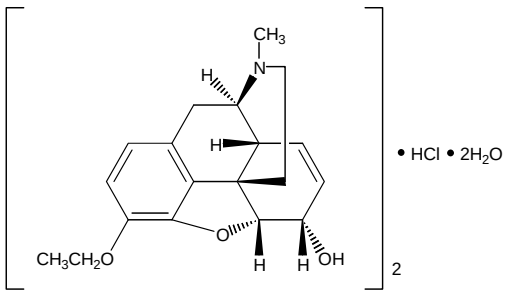
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weight accurately about 0.25 g of Ethyl Aminobenzoate, previously dried, dissolved in 10 mL of hydrochloric acid and 70 mL of water, add 10 mL of a solution of potassium bromide (3 in 10) and cool to a temperature below 15 ºC. Then titrate with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration, Endpoint Detection Methods in Titrimetry).

Each mL of 0.1 mol/L sodium nitrite VS = 16.519 mg of $C_9H_{11}NO_2$

Containers and Storage ***Containers***—Well-closed containers.

Ethylmorphine Hydrochloride Hydrate



$C_{19}H_{23}NO_3 \cdot HCl \cdot 2\,H_2O$: 385.88

(4*R*,4a*R*,7*S*,7a*R*,12b*S*)-9-Ethoxy-3-methyl-2,4,4a,7,7a,13-hexahydro-1*H*-4,12-methanobenzofuro[3,2-e]isoquinoline-7-ol hydrochloride dihydrate [*6746-59-4*]

Ethylmorphine Hydrochloride Hydrate, when dried, contains not less than 98.0 % and not more than 101.0 % of ethylmorphine hydrochloride ($C_{19}H_{23}NO_3 \cdot HCl$: 349.85).

Description Ethylmorphine Hydrochloride Hydrate appears as white to pale yellow crystals or crystalline powder.
Ethylmorphine Hydrochloride Hydrate is very soluble in methanol or in acetic acid (100), freely soluble in water, soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in ether.
Ethylmorphine Hydrochloride Hydrate is colored by light.

Melting point—About 123 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Ethylmorphine Hydrochloride Hydrate and Ethylmorphine Hydrochloride Hydrate RS, separatey, in water (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ethylmorphine Hydrochloride Hydrate and Ethylmorphine Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Ethylmorphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -103 ~ -106° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH Dissolve 0.10 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity ***Related substances***—Dissolve 0.20 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of diluted ethanol (1 in 2) and use this solution as the test solution. Pipet 0.5 mL of the test solution, add diluted ethanol (1 in 2) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated ethanol, toluene, acetone and ammonia solution (28) (14 : 14 : 7 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Water 8.0 ~ 10.0 % (0.25 g, volume titration, direct titration).

Residue on Ignition Not more than 0.1 % (0.5 g).

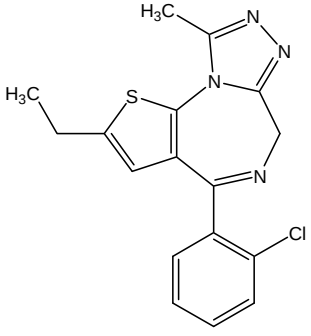
Assay Weigh accurately about 0.5 g of Ethylmorphine Hydrochloride Hydrate and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.985 mg of $C_{19}H_{23}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Etizolam



$C_{17}H_{15}ClN_4S$: 342.85

7-(2-Chlorophenyl)-4-ethyl-13-methyl-3-thia-1,8,11,12- tetraazatricyclo[8.3.0.02,6}]trideca-2(6),4,7,10,12-pentaene [*40054-69-1*]

Etizolam contains not less than 98.5 % and not more than 101.0 % of etizolam ($C_{17}H_{15}ClN_4S$).

Description Etizolam appears as white to pale yellowish white crystalline powder.
Etizolam is soluble in ethanol (99.5), sparingly soluble in acetonitrile and in acetic anhydride, and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Etizolam and Etizolam RS in ethanol (99.5) (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Etizolam and Etizolam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting point 146 ~ 149 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Etizolam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 20 mg of Etizolam in 50 mL of acetonitrile, and use this solution as the test solution. Pipet 1 mL of the test solution, and add acetonitirle to make exactly 20 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etizolam obtained from the test solution is not larger than the peak area of etizolam from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of etizolam obtained from 10 μL of this solution is equivalent to 8 to 12 % of that from the standard solution.

System performance: Dissove 0.02 g each of Etizolam and ethyl parahydroxybenzoate in the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ethyl parahydroxybenzoate and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2 %,

Time span of measurement: About 5 times as long as the retention time of etizolam beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

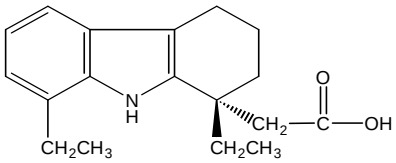
Assay Weigh accurately about 0.3 g of Etizolam, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). The end point is the second inflection point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.14 mg of $C_{17}H_{15}ClN_4S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Etodolac



and enantiomer

$C_{17}H_{21}NO_3$: 287.35

2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid [*41340-25-4*]

Etodolac contains not less than 98.0 % and not more

than 102.0 % of etodolac ($C_{17}H_{21}NO_3$), calculated on the anhydrous basis.

Description Etodolac appears as white to pale yellow crystals or crystalline powder.
Etodolac is freely soluble in methanol or in ethanol (99.5), and practically insoluble in water.
A solution of Etodolac in methanol (1 in 50) shows no optical rotation.

Melting point—About 147 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Etodolac and Etodolac RS in ethanol (99.5) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Etodolac and Etodolac RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Chloride***—Dissolve 0.5 g of Etodolac in 30 mL of methanol, add water to make exactly 50 mL, and use this solution as the test solution. Dissolve 0.42 mL of 0.01 mol/L hydrochloric acid VS in 30 mL of methanol, add water to make exactly 50 mL, and use this solution as a control solution (not more than 0.03 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Etodolac according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Limit of methanol and ethanol***—Dissolve about 0.5 g of Etodolac, weighed accurately, in 5.0 mL of the internal standard solution, and use this solution as the test solution. Separately, pipet 5 mL each of methanol and ethanol (95), add dimethylformamide to make exactly 200 mL, pipet 5.0 mL of this solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 10.0 mL of the standard stock solution, add 5.0 mL of the internal standard, dilute with *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of methanol and ethanol in Etodolac to that of the internal standard for the test solution and the standard solution, respectively. Amount of methanol and ethanol in the portion of Etodolac is not more than 0.1 %, respectively.

$$\text{Amount (\%) of methanol or ethanol} = 500 \times \frac{C}{W} \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of ethanol or methanol in the standard solution.

W: Weight (mg) of etodolac in the test solution.

Internal standard solution—Dissolve a suitable quantity of 2-propanol in *N,N*-dimethylformamide to make a solution having a concentration of 2.5 μL/mL. Pipet 5.0 mL of this solution, and dilute this solution with *N,N*-dimethylformamide to make exactly 100 mL.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica capillary column, 0.32 mm in internal diameter and 25 m in length, coated with a 5 μm film of 1 % vinyl–5 % phenylmethylpolysiloxane for gas chromatography.

Carrier gas: Helium.

Flow rate: 50 mL/minute.

Injection port temperature: 200 ºC.

Detector temperature: 300 ºC.

Column temperature: Maintain the temperature of 45 ºC for 5 minutes, then raise to 280 ºC at the rate of 30 ºC per minute, and maintain at 280 ºC for 27 minutes.

System suitability

System performance: When the procedure is run with 1 μL of the standard solution, as directed under the above operating conditions, methanol, ethanol, and 2-propanol are eluted in this order with the resolution between their peaks being not less than 1.0.

(4) ***Related substances***—Dissolve 25 mg of Etodolac in acetonitrile to make exactly 250 mL, and use this solution as the test solution. Perform the test with the test solution as directed under Liquid Chromatography according to the following conditions. Each peak area of any peak other than the principal peak from the test solution is not more than 0.5 percent to the total area of all peaks, and the total area is not more than 2.0 percent.

$$\text{Content (\%) of related substances} = 100 \times \frac{A_T}{A_S}$$

A_T: Each peak area of the peaks other than the principle peak.

A_S: Total area of all peaks.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (3 to 10 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as follows. First 5 minutes, use mixture of 60 percent solution A and 40 percent solution B as the mobile phase. For next 30 minutes, change the mixture ratio as linear gradient to finally make mixture of 20 percent solution A and 80 percent solution B. Adjust mobile phase to 40 percent

of solution B before injection of the test solution and the standard solution, and keep re-equilibrium.

Mobile phase A: Mix 0.6 mL of phosphoric acid with 100 mL of water.

Mobile phase B: Mix 0.6 mL of phosphoric acid with 100 mL of acetonitrile.

Flow rate: 1 mL/min.

System suitability

System performance: Dissolve suitable amount each of Etodolac Related Substance I RS and Etodolac RS in acetonitrile to make solutions containing 10 μg of Etodolac Related Substance I RS and 0.2 mg of Etodolac RS per mL, respectively. When the procedure is run with 20 μL of this solution, as directed under the above operating conditions, etodolac related substance I and Etodolac are eluted in this order with the resolution between their peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the solution for system performance, as directed under the above operating conditions, the relative standard deviation of the ratio of the peak area is not more than 3 %.

Water Not more than 0.5 % (2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.23 g of Etodolac, dissolve in 60 mL of methanol, and titrate with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS = 28.736 mg of $C_{17}H_{21}NO_3$

Containers and Storage ***Containers***—Tight containers.

Etodolac Tablets

Etodolac Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of etodolac ($C_{17}H_{21}NO_3$: 287.36)

Method of Preparation Prepare as directed under Tablets, with Etodolac.

Identification Test The retention time of major peak of the test solution corresponds to that of the standard solution as obtained in the Assay.

Dissolution Test Take 1 tablet of Etodolac Tablets, perform the test as directed in Method 1 under the Dissolution Test at 100 revolutions per minute, and using 1000 mL of pH 6.8 phosphate buffer as the dissolution solution. After 30 minutes from starting of the test, take 20 mL of the dissolved solution, and filter through a membrane filter with a pore size of 0.8 μm or less. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Seperately weigh accurately 25 mg of Etodolac RS, and dissolve in the dissolution solution to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution solution to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the maximum wavelength at about 274 nm as directed under Ultraviolet-visible Spectrophotometry using the dissolution solution as a blank. The dissolution rate of Etodolac Tablets after 30 minutes should be not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of etodolac } (C_{17}H_{21}NO_3) = W_S \times \frac{A_T}{A_S} \times \frac{100}{C}$$

W_S: Amount (mg) of Etodolac RS

C: Labeled amount (mg) of etodolac ($C_{17}H_{21}NO_3$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Take 1 tablet of Etodolac Tablets, and shake with 10 mL of phosphate buffer until the tablet is disintegrated. Add phosphate buffer to make exactly 100 mL, and centrifuge this solution. Pipet x mL of the supernatant liquid, add phosphate buffer to make exactly v mL to provide a solution that contains about 25 μg of etodolac ($C_{17}H_{21}NO_3$) per mL, and use this solution as the test solution. Separately, weigh accurately 25 mg of etodolac reference standard, and dissolve in phosphate buffer to make exactly 100 mL. Pipet 10 mL of this solution, add phosphate buffer to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at maximum wavelength at about 274 nm as directed under the Ultraviolet-visible spectrophotometry.

$$\text{Amount (mg) of etodolac } (C_{17}H_{21}NO_3) = \text{amount (mg) of Etodolac RS} \times \frac{A_T}{A_S} \times \frac{V}{10} \times \frac{1}{x}$$

Assay Weigh accurately and powder not less than 20 Etodolac Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of etodolac ($C_{17}H_{21}NO_3$), and add 30 mL of a mobile phase. Shake for 15 minutes, and sonicate 5 minutes for integration. After cooling, add the mobile phase to make exactly 50 mL. After leaving 10 minutes, pipet 10.0 mL of this solution, then add the mobile phase to make exactly 100 mL, filter, and use this filtrate as the test solution. Separately, weigh exactly 20 mg Etodolac RS and dissolve in the mobile phase to make exactly 100 mL, and

use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of each solution.

$$\text{Amount (mg) of etodolac } (C_{17}H_{21}NO_3) = \text{amount (mg) of Etodolac RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column, about 4.6 nm in internal diameter and about 25 cm in length, packed with 3 ~ 10 μm in particle diameter octadecylsilanized silicagel for liquid chromatography.

Mobile phase: acetonitrile · water · phosphate mixture (500 : 500 : 0.25).

Flow rate: 1.5 mL/minute.

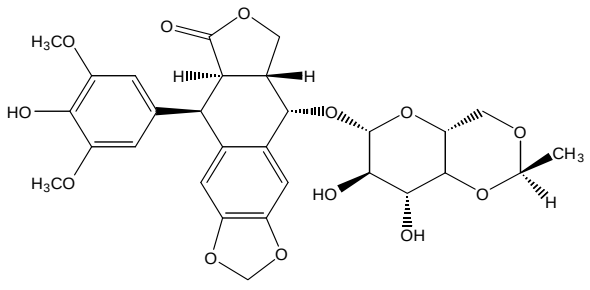
System suitability

System performance: Dissolve Etodolac related substance Ⅰ RS and Etodolac RS in acetonitrile to make Etodolac related substance Ⅰ RS 10 μg and Etodolac RS about 0.2 mg per mL. When the precedure is run with 20 μL of this solution, as directed under the above operating conditions, Etodolac related substance Ⅰ and Etodolac are eluted in this order with the resolution between their peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak areas is not more than 3 %.

Containers and Storage ***Containers***—Tight containers.

Etoposide



$C_{29}H_{32}O_{13}$: 588.56

(5*S*,5a*R*,8a*R*,9*R*)-5-[[(2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7,8-Dihydroxy-2-methyl-4,4a,6,7,8,8a-hexahydropyrano[3,2-d][1,3]dioxin-6-yl]oxy]-9-(4-hydroxy-3,5-dimethoxyphenyl)-5a,6,8a,9-tetrahydro-5*H*-[2]benzofuro[6,5-f][1,3]benzodioxol-8-one

[*33419-42-0*]

Etoposide contains not less than 98.0 % and not more than 102.0 % of etoposide ($C_{29}H_{32}O_{13}$), calculated on the anhydrous basis.

Description Etoposide appears as white crystals or crystalline powder.

Etoposide is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly in water.

Melting point—about 260ºC

Identification (1) Determine the absorption spectra of solutions of Etoposide and Etoposide RS, respectively, in methanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Etoposide and Etoposide RS, according to the paste method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: −100 ~ −105° [0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm].

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Etoposide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 50 mg of Etoposide in 10 mL of methanol and add mobile solution to make 50 mL and use this solution as the test solution. Pipet 2 mL of the test solution, add the mobile phase to make 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of any peak other than etoposide obtained with the test solution is not greater than 1/5 times the peak area of etoposide obtained with the standard solution, and the total area of all peaks other than the peak of etoposide obtained with the test solution is not larger than 1/2 times the peak area of etoposide obtained with the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for the required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of etoposide obtained with 50 μL of this solution is equivalent to 7 to 13 % of that of etoposide obtained

with 50 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etoposide is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of etoposide beginning after the solvent peak.

Water Not more than 4.0 % (0.5 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 25 mg each of Etoposide and Etoposide RS (determined previously the water), dissolve separately in and dilute with methanol to make exactly 25 mL each. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make exactly 50 mL and use this solution as the test solution and standard solution, respectively. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the ratios, Q_T and Q_S, of the peak area of etoposide to that of the internal standard.

$$\text{Amount (mg) of } C_{29}H_{32}O_{13} = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of the Etoposide RS, calculated on the anhydrous basis

Internal standard solution—A solution of 2,6-dichlorophenol in methanol (3 in 2500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with phenylsilyl silica gel for liquid chromatography (10 μm particle diameter).

Column temperature: A constant temperature of about 35 ºC

Mobile phase: Dissolve 6.44 g of sodium sulfate decahydrate in diluted acetic acid (100) (1 in 100) to make 1000 mL, and add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retentiontime of etoposide is about 20 minutes.

System suitability

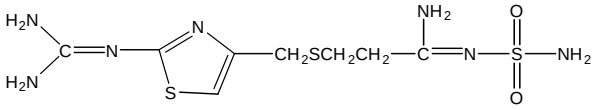
System performance: Dissolve 10 mg of Etoposide in 2 mL of methanol, add 8 mL of the mobile phase, and mix well. Add 0.1 mL of diluted acetic acid (100) (1 in 25) and 0.1 mL of phenolphthalein TS, and add sodium hydroxide TS until the color of the solution changes to faintly red. After allowing to stand for 15 minutes, add 0.1 mL of diluted acetic acid (100) (1 in 25). When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 1.3 with respect to etoposide is not less than 3.0.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of etoposide to that of internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Famotidine



$C_8H_{15}N_7O_2S_3$: 337.45

3-[({2-[(Diaminomethylidene)amino]-1,3-thiazol-4-yl}methyl)sulfanyl]-*N*′-sulfamoylpropanimidamide [*76824-35-6*]

Famotidine, when dried, contains not less than 98.5 % and not more than 101.0 % of famotidine ($C_8H_{15}N_7O_2S_3$).

Description Famotidine appears as white to yellowish white crystals.

Famotidine is freely soluble in acetic acid (100), slightly soluble in ethanol (95), and very slightly soluble in water.

Famotidine dissolves in 0.5 mol/L hydrochloric acid TS.

Famotidine is gradually colored by light.

Melting point—About 164 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Famotidine and Famotidine RS in 0.05 mol/L monobasic potassium phosphate TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Famotidine and Famotidine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Famotidine in 10 mL of 0.5 mol/L hydrochloric acid TS: the solution is clear and colorless to pale

yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Famotidine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.20 g of Famotidine in 10 mL of acetic acid (100) and use this solution as the test solution. Pipet 1 mL of the test solution and add acetic acid (100) to make exactly 100 mL. Pipet 1 mL, 2 mL and 3 mL of this solution, add acetic acid (100) to make exactly 10 mL, respectively and use these solutions as the standard solutions (1), (2) and (3). Perform the test with the test solution and the standard solutions (1), (2) and (3) as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solutions (1), (2) and (3) on a plate of silica gel (5 to 7 μm in particle diameter) with fluorescent indicator for thin-layer chromatography and dry in a stream of nitrogen. Develop the plate with a mixture of ethyl acetate, methanol, toluene and ammonia solution (28) (40 : 25 : 20 : 2) to a distance of about 8 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot of the starting point from the test solution are not more intense than the spot from the standard solution (3). Total intensity of the spots other than the principal spot and other than the spot of the starting point from the test solution is not more than 0.5 % calculated on the basis of intensities of the spots from the standard solutions (1) and (2).

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 80 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Famotidine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.873 mg of $C_8H_{15}N_7O_2S_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Famotidine for Injection

Famotidine for injection is a solution for injection which is dissolved before use. Famotidine for injection contains not less than 94.0 % and not more than 106.0 % of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of Preperation Prepare as directed under Injections, with Famotidine.

Description Famotidine for injection occurs as porous masses or powder.

Identification Weigh a portion of Famotidine for injection, equivalent to 10 mg of Famotidine according to the labeled amount, dissolve in 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, to 5 mL of this solution add 0.05 mol/L potassium dihydrogen phosphate TS to make 50 mL, and determine the spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits the maximum absorbance between 263 nm and 267 nm.

pH Weigh a portion of Famotidine for injection, equivalent to 20 mg of famotidine according to the labeled amount, dissolve in 1 mL of water: the pH of this solution is between 4.9 and 5.5.

Purity (1) ***Clarity and color of solution***— Weigh a portion of Famotidine for injection, equivalent to 20 mg of famotidine according to the labeled amount, dissolve in 1 mL of water; the solution is clear and colorless.

(2) ***Related substances***—Take a number of Famotidine for injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the major peak from the test solution is not larger than the major peak from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and system performance: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of famotidine obtained from 5 μL of the standard solution is between 5 mm and 10 mm.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of famotidine obtained from 5 μL of this solution is equivalent to 8 to 12 % of that from the standard solution.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the

above operating conditions, the relative standard deviation of the peak rea of famotidine is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of famotidine after the solvent peak.

Water Not more than 1.5 % (0.1 g, coulometric titration).

Sterility Test It meets the requirement.

Baterial Endotoxins Less than 15 EU/mg of Famotidine for injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Take a number of Famotidine for injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL. Pipet 5 mL of this solution, add 5.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Famotidine RS, previously dried in vacuum with P_2O_5 at 80 °C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution add 5.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

$$\text{Amount (mg) of famotidine } (C_8H_{15}N_7O_2S_3) = \text{Amount (mg) of Famotidine RS} \times \frac{Q_T}{Q_S} \times 2$$

Internal standard solution—Take 5 mL of a solution of methyl paraoxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust the pH to 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution, add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability

System performance: When the precedure is run with 5 μL of the standard solution, as directed under the above operating conditions, famotidine and internal standard are eluted in this order with the resolution between their peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Famotidine Tablets

Famotidine Tablets contain not less than 94.0 % and not more than 106.0 % of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of Preperation Prepare as directed under Tablets, with Famotidine.

Identification Weigh a portion of powdered Famotidine Tablets, equivalent to 10 mg of famotidine according to the labeled amount, add 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, shake well and centrifuge. Take 5 mL of the clear supernatant liquid, add 0.05 mol/L potassium dihydrogen phosphate TS to make 50 mL, and determine the spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits an absorbance maximum between 263 nm and 267 nm.

Purity ***Related substances***—To not less than 10 Famotidine Tablets add 200 mL of the diluent, and shake well to disintegrate. To this solution add 200 mL of methanol, mix at 300 revolutions per minute for one hour, add the diluent to make exactly 1000 mL, and filter. Pipet an amount of the filtrate, equivalent to 10 mg of famotidine, add the diluent to make 100 mL, and use this solution as the test solution. Separately, weigh accurately 10 mg of Famotidine RS, add 20 mL of methanol, sonicate for 5 minutes, add the diluent to mak exactly 100 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography. Determine each peak area of each solution by the automatic integration method, and

calculate the amount of each related substance from the test solution: famotidine related substance I {3-[2-(diaminomethyleneamino)-1,3-thiazole-4-ylmethylfulfinyl]-*N*-sulfamoyl-propanamidine} is not more than 1.0 %, famotidine related substance II {3-[2-(diaminomethyleneamino)-1,3-thiazole-4-ylmethylthio]-propanoic acid}, famotidine related substance III {3-[2-(diaminomethyleneamino)-1,3-thiazole-4-ylmethylthio]-*N*-sulfamoyl-propanamide}, and famotidine related substance IV {3-[2-(diaminomethyleneamino)-1,3-thiazole-4-ylmethylthio]-propanamide} are not more than 0.5 %, respectively, and the total amont of related substances is not more than 1.5 %. Use the peak area of famotidine related substance IV, determined by the automatic integration method, after dividing by its relative response factor, 1.3.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C \times N} \times \frac{A_i}{A_S}$$

C_S: Concentration (mg/mL) of famotidine in the standard solution

C: Labeled amount (g) of famotidine in 1 tablet

N: Number of tablets used to prepare the test solution

A_i: Peak area of each related substance from the test solution

A_S: Peak area of famotidine from the standard solution

Diluent—Dissolve 6.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 with 1 mol/L potassium hydroxide, and add water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of the buffer solution and acetonitrile (93 : 7)

Flow rate: About 1.4 mL/minute

System suitability

Test for required detectability: To 10 mg of famotidine add 1 mL of 0.1 mol/L hydrochloric acid, heat at 80 °C for 30 minutes, and cool to room temperature. To this solution add 2 mL of 0.1 mol/L sodium hydroxide TS, heat at 80 °C for 30 minutes, cool to room temperature, neutralize with 1 mL of 0.1 mol/L hydrochloric acid, and add the diluent to make 50 mL. Pipet 10 mL of this solution, add to a solution prepared by dissolving 5 mg of famotidine in 8 mg of methanol, and add the diluent to make 50 mL. To 25 mL of this solution add the diluent to make 50 mL, and use this solution as the system suitability stock solution. To 1 to 1.5 mL of this solution add 1 drop of hydrogen peroxide TS, and use this solution as the system suitability solution. When the procedure is run with 50 μL of this solution under the above operating conditions, the relative retention times of famotidine related substances I, II, III, and IV are 0.4, 0.7, 0.8, and 1.2, respectively.

System performance: When the procedure is run with 50 μL of the system suitability solution under the above operating conditions, the resolutions between the peaks of famotidine related substance III and famotidine and between the peaks of famotidine and famotidine related substance IV are not less than 1.3, respectively, and the capacity factor of the peak of famotidine is not less than 2.0.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation is not more than 2.0 %.

Buffer solution—Dissolve 13.6 g of sodium acetate trihydrate in 750 mL of water, add 1 mL of triethylamine, adjust the pH to 6.0 with acetic acid (100), and add water to make 1000 mL.

Dissolution Test Perform the test with 1 tablet of Famotidine Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of 0.1 mol/L phosphate buffer (pH 4.5) as the dissolution solution. Take 20 mL of the dissolved solution at 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately 20 mg of Famotidine RS, add buffer solution to make exactly 100 mL. Pipet 5.0 mL of this solution, add the buffer solution to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at an absorption maximum near 265 nm as directed under Ultraviolet-visible Spectrophotometry.

The dissolution rate of Famotidine Tablets in 30 minutes should be not less than 75 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of famotidine } (C_8H_{15}N_7O_2S_3) = \text{Amount (mg) of Famotidine RS} \times \frac{A_T}{A_S} \times \frac{90}{C}$$

C: labeled amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the Content Uniformity Test is performed according to the following method

Take 1 tablet of Famotidine Tablets, add 2 mL of water, shake to disintegrate. Add a suitable amount of methanol and shake well, then add methanol to make exactly V mL of a solution containing about 0.2 mg of fa-

motidine ($C_8H_{15}N_7O_2S_3$) per mL and centrifuge. Pipet 10 mL of the clear supernatant liquid, add 2.0 mL of the internal standard solution, add the mobile phase to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Famotidine RS, previously dried in vacuum with P_2O_5 at 50 °C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add 2.0 mL of the internal standard solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions described in the Assay and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

$$\text{Amount (mg) of famotidine } (C_8H_{15}N_7O_2S_3) = \text{Amount (mg) of Famotidine RS} \times \frac{Q_T}{Q_S} \times \frac{V}{500}$$

Internal standard solution—Take 5 mL of a solution of methyl paraoxybenzoate in methanol (1 in 500) add water to make 50 mL.

Assay Take a number of Famotidine Tablets, equivalent to 0.2 g of famotidine ($C_8H_{15}N_7O_2S_3$), add 50 mL of water and disintegrate by shaking well. Then add 100 mL of methanol, shake well, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the clear supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Famotidine RS, previously dried in vacuum with P_2O_5 at 80 °C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

$$\text{Amount (mg) of famotidine } (C_8H_{15}N_7O_2S_3) = \text{Amount (mg) of Famotidine RS} \times \frac{Q_T}{Q_S} \times 2$$

Internal standard solution—Take 5 mL of a solution of methyl paraoxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust the pH to 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution, add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

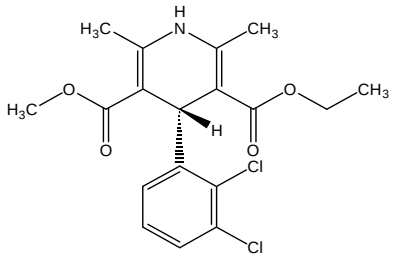
System suitability

System performance: When the precedure is run with 5 μL of the standard solution, as directed under the above operating conditions, famotidine and internal standard are eluted in this order with the resolution between their peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Felodipine



and enantiomer

$C_{18}H_{19}Cl_2NO_4$: 384.25

3-Ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate [*72509-76-3*]

Felodipine contains not less than 98.0 % and not more than 101.0 % of felodipine ($C_{18}H_{19}Cl_2NO_4$), calculated on the dried basis.

Description Felodipine is a pale yellow to yellow crystalline powder.

Felodipine is freely soluble in acetone or in ethanol (95), very slightly soluble in heptane, and practically insoluble in water.

Identification (1) Determine the infrared spectra of Felodipine and Felodipine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensity of absorption at the same wavenumbers.

(2) The retention time of the major peak in the chromatogram of the test solutoin is same as that of the standard solution, as obtained in the Assay.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Felodipine in methanol to make exactly 50 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, using methanol as the blank: the absorbance at 440 nm is not more than 0.2.

(2) ***Heavy metals***—Proceed with 1.0 g of Felodipine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform the test with 40 μL of the test solution prepared as in the Assay as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the test solution: each peak area except major peak to that of major peak area is not more than 1.0 %, and the sum of all peak area is not more than 1.5 %.

$$\text{Amount (\%) of related substances} = 100 \times \frac{A_i}{A_s}$$

A_i : each peak area except major peak
A_s : the sum of all peak area

Operating conditions

Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of felodipine.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 30 mg each of Felodipine and Felodipine RS, add the mobile phase to make exactly 100 mL, and use these solutions as the test solution and the standard solution. The test solution and the standard solution are prepared before use. Perform the test with 40 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine A_T and A_S, of the peak area of felodipine of each solution.

$$\text{Amount (mg) of felodipine } (C_{18}H_{19}C1_2NO_4) = \text{Amount (mg) of Felodipine RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer, acetonitrile, and methanol (2 : 2 : 1).

Flow rate: 1 mL/minute.

System suitability

System performance: Dissolve 0.15 g of Felodipine in a mixture of 25 mL of *t*-butyl alcohol and 25 mL of 1 mol/L perchloric acid, add 10 mL of 0.1 mol/L ceric sulfate, mix, and allow to stand for 15 minutes. Add 3.5 mL of 10 mol/L sodium hydroxide TS, and neutralize with 2 mol/L sodium hydroxide TS. Shake the mixture with 25 mL of methylene chloride in a sparator. Draw off the lower layer, and evaporate it to dryness under a stream of nitrogen on a water-bath. Dissolve 10 mg of the residue (felodipine oxidation product) and 5 mg of Felodipine RS in the mobile phase to make 100 mL. Pipet 1.0 mL of this solution, and add mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution, as directed under the above operating conditions, felodipine oxidation product and felodipine are eluted in this order with the resolution between their peaks being not less than 2.5. And perform the test with 40 μL of the standard solution, as directed under the above operating conditions, the symmetry factor of the peak of felodipine is not more than 1.5.

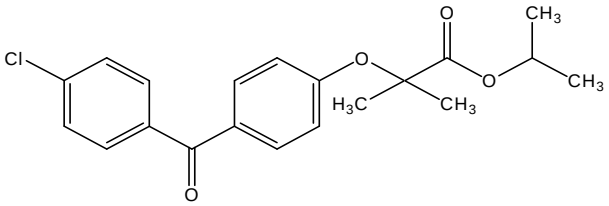
Detection sensitivity: Adjust the detection sensitivity so that the heights of the two peaks in the chromatogram are not less than 20 % of the full scale.

Phosphate buffer—Dissolve 6.9 g of sodium dihydrogen phosphate dihydrate in 400 mL of water, add 8 mL of 1 mol/L phosphoric acid, and add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fenofibrate



$C_{20}H_{21}ClO_4$: 360.83

Propan-2-yl 2-{4-[(4-chlorophenyl)carbonyl] phenoxy}-2-methylpropanoate [*49562-28-9*]

Fenofibrate contains not less than 98.5 % and not more than 101.0 % of fenofibrate ($C_{20}H_{21}ClO_4$), calculated on the dried basis.

Description Fenofibrate is a white crystalline powder. Fenofibrate is very soluble in dichloromethane, slightly soluable in ethanol (95) and practically insoluable in water.

Identification Determine the infrared spectra of Fenofibrate and Fenofibrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 79 ~ 82 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.50 g of Fenofibrate in aceton to make exactly 10 mL: the solution is clear.

(2) ***Acid***—Dissolve 1.0 g of Fenofibrate in 50 mL of ethanol, previously neutralized using 0.2 mL of phenolphthalein solution TS: not more than 0.2 mL of 0.1 mol/L sodium hydroxide VS is required to change the color of the indicator to pink.

(3) ***Chloride***—To 0.5 g of Fenofibrate, add 25 mL of water, heat at 50 ºC for 10 minutes, cool and filter. To 5 mL of the filtrate, add 6 mL of dilute nitric acid TS and add water to make 50 mL. Prepare the control solution with 6 mL of dilute nitric acid and 0.30 mL of 0.01 mol/L hydrochloric acid VS, adding water to make 50 mL (not more than 0.01 %).

(4) ***Sulfate***— To 0.5 g of Fenofibrate, add 25 mL of water, heat at 50 ºC for 10 minutes, cool and filter. To 10 mL of the filtrate, add 1 mL of dilute hydrochloric acid TS and add water to make 50 mL. Prepare the control solution with 1 mL of dilute hydrochloric acid and 0.42 mL of 0.005 mol/L sufuric acid VS, adding water to make 50 mL (not more than 0.01 %).

(5) ***Heavy metals***—Proceed with 1.0 g of Fenofibrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Related substances***—Disolve 0.100 g of Fenofibrate in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 25.0 mg of Fenofibrate RS in the mobile phase to make exactly 25 mL and use this solution as the standard solution (1). And separately, dissolve 10.0 mg of Fenofibrate RS, 10.0 mg of fenofibrate related substance I RS [(4-chlorophenyl)(4-hydroxyphenyl) methanone], 10.0 mg of of fenofibrate related substance II RS [2-[4-(4-chloro benzoyl)phenoxy]-2-methylpropanoic acid (fenofibric acid)] and 20.0 mg of fenofibrate related substance III RS [1-methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl] oxy]2-methylpropanoate] in the mobile phase to make exactly 100 mL. To 1.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following conditions: the areas of the peaks of fenofibrate related substance I, II and III from the test solution is not larger than the areas of the corresponding peaks from the standard solution (2) (0.1 % each of fenofibrate related substance I and II; 0.2 % of fenofibrate related substance III). The areas of peaks other than the major peak and peaks of fenofibrate related substance I, II and III from the test solution is not larger than the the area of peak of fenofibrate from the standard solution (2) (0.1 %). The total area of all the peaks other than the major peak from the test solution is not larger than 5 times area of the peak of fenofibrate from the standard solution (2) (0.5 %). Disregard any peak with an area less than 0.l times that of the peak of fenofibrate from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 286 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and water (adjust the pH to 2.5 with phosphoric acid) (70 : 30).

Flow rate: 1 mL/minute.

System suitability

System performance: Adjust the detection sensitivity with the standard solution (2) so that the heights of the peaks in the chromatogram are not less than 20.0 % of the full scale. The relative retention times of fenofibrate related substances I, II, IV, V, VI, VII and III are about 0.34, 0.36, 0.50, 0.65, 0.80, 0.85 and 1.35, respectively and the resolution between the peaks of fenofibrate related substances I and II is not less than 1.5.

Time span of measurement: About 2 times as long as the retention time of fenofibrate, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (0.5 g, 60 ºC, in vacuum, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh acculately about 25 mg each of Fenofibrate and Fenofibrate RS, dissolve in exactly 25 mL of the mobile phase and use these solutions as the test solution and the standard solution (1), respectively. Seperately, weigh 10.0 mg of Fenofibrate RS, 10.0 mg of fenofibrate related compound II RS and 20.0 mg of fenofibrate related compound III RS, dissolve in the mobile phase to make exactly 100 mL, take 1.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with 5 μL each of the test solution and the standard solution (1) as directed under Liquid Chromatography according to the following conditions of the Related substances, and determine the peak areas, A_T and A_S, of fenofibrate of each solution.

$$\text{Amount (mg) of fenofibrate } (C_{20}H_{21}ClO_4) = \text{Amount (mg) of Fenofibrate RS} \times \frac{A_T}{A_S}$$

System suitability

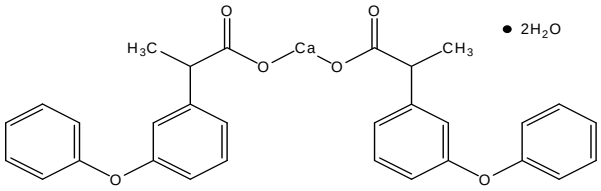
Detection sensitivity: Adjust the detection sensitivity so that the heights of the peaks in the chromatogram are not less than 50.0 % of the full scale.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution (1) as directed under the above operating conditions, the relative standard deviation of the peak area of fenofibrate is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fenoprofen Calcium Dihydrate



$C_{30}H_{26}CaO_6 \cdot 2H_2O$: 558.63

Calcium 2-(3-phenoxyphenyl)propanoate dihydrate [*152864-45-4*]

Fenoprofen Calcium Dihydrate contains not less than 97.0 % and not more than 103.0 % of fenoprofen calcium ($C_{30}H_{26}CaO_6$: 522.60), calculated on the anhydous basis.

Description Fenoprofen Calcium Dihydrate appears as white crystalline powder.

Fenoprofen Calcium Dihydrate is slightly soluble in n-hexanol, in methanol, or in water and practically insoluble in chloroform.

Identification (1) Mix 1 g of Fenoprofen Calcium Dihydrate with 50 mL of acetic acid (31), heat and filter. Add 2 mL of ammonium oxalate TS to the filtrate: white precipitate is formed and it dissolves in 3 mol/L hydrochloric acid TS.

(2) Determine the infrared spectra of Fenoprofen Calcium Dihydrate and of Fenoprofen Calcium Dihydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Calcium***—Weigh accurately about 0.75 g of Fenoprofen Calcium Hydrate, dissolve in ethanol (95) by heating, if necessary, add ethanol (95) to make exactly 50 mL and use this solution as the test solution. Put 70 mL of water, 2 mL of a solution of sodium hydroxide (1 in 10) and 0.3 g of hydroxynaphtol, mix, add about 1 mL of the test solution and titrate with 0.05 mol/L disodium ehthylenediaminetetraacetate VS until blue color appears (7.3 to 8.0 %, calculated on the anhydrous basis)

Each mL of 0.05 mol/L
disodium ethylenediaminetetra acetate VS
= 2.044 mg of Ca

(2) ***Heavy metals***—Proceed with 1.0 g of Fenoprofen Calcium Dihydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve about 0.2 g of Fenoprofen Calcium Hydrate, accurately weighed, in a mixture of water-acetonitrile (1 : 1) to make exactly 100 mL and use this solution as the test solution. Separately, dissolve about 20 mg of Fenoprofen Calcium Dihydrate RS, accurately weighed, in a mixture of water-acetonitrile (1 : 1) to make exactly 100 mL, take 5.0 mL of this solution, add a mixture of water-acetonitrile (1 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of the test solution and the standard solution by the automatic integration and calculate the amount of each related compound: the amount of each related compound is not more than 0.5 % and the total amount of related compounds is not more than 2.0 %.

$$\text{Amount (\%) of a related substance} = 10000 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

C: Concentration (mg/mL) of Fenoprofen Calcium Dihydrate RS in the standard solution

W: Amount (mg) of Fenoprofen Calcium Dihydrate taken to prepare the test solution

A_i: Peak area of each related compound obtained from the test solution

A_s: Peak area of fenoprofen obtained from the standard solution

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 270 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase : With the mobile phase A and the mobile B, control the composition stepwise or gradiently as follows.

Mobile phase A: A mixture of water and acetic acid (31) (98 : 2)

Mobile phase B: A mixture of acetonitrile and acetic acid (31) (98 : 2)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	70	30
0-3	70	30
3-41	70→10	30→90
41-42	10	90
42-43	10→70	90→30
43-55	70	30

Flow rate: 1.5 mL/minute
System suitability

System performance: Dissolve 2 mg of 3-phenoxybenzoic acid and Fenoprofen Calcium Dihydrate RS in a mixture of water and acetonitrile (1 : 1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, relative retention times are about 0.89 for 3-phenoxybenzoic acid and 1.00 for fenoprofen, the resolution between them is not less than 9.0 and the symmetry factor of fenoprofen is not more than 2.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fenoprofen is not more than 2.0 %.

Water 5.0 ~ 8.0 % (1 g, volumetric titration, direct titration)

Assay Weigh accurately about 70 mg each of Fenoprofen Calcium Dihydrate and Fenoprofen Calcium Dihydrate RS, add 0.5 mL of 0.5 mol/L hydrochloric acid TS and 2 mL of ethanol, add a mixture of methanol and water (7 : 3) to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, measure the peak areas, A_T and A_S, of fenopropen obtained from each solutions.

$$\text{Amount (mg) of fenoprofen calcium } [(C_{15}H_{13}O_3)_2Ca] = 100 \times C \times \frac{A_T}{A_S}$$

C : Amount (mg) of fenoprofen calcium in Fenoprofen Calcium Dihydrate RS, calculated on the anhydrous basis

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 272 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase : A mixture of acetonitrile, water and phosphoric acid (50 : 49.6 : 0.4)

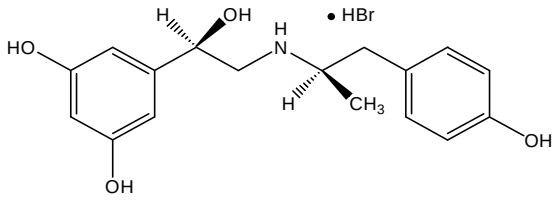
Flow rate: 2 mL/minute
System suitability

System performance: Dissolve 5 mg of Fenoprofen Calcium Dihydrate RS and 5 mg of gemfibrozil in a mixture of methanol and water (1 : 1) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, relative retention times are about 0.5 for fenoprofen and 1.0 for gemfibrozil, the resolution between them is not less than 8 and the column efficiency determined from the peak of fenoprofen is not less than 3000 theoretical plates.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fenoprofen is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Fenoterol Hydrobromide



$C_{17}H_{21}NO_4 \cdot HBr$: 384.27

5-[1-Hydroxy-2-[1-(4-hydroxyphenyl)propan-2-ylamino]ethyl]benzene-1,3-diolhydrobromide
[*1944-12-3*]

Fenoterol Hydrobromide contains not less than 99.0 % and not more than 101.0 % of fenoterol hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$), calculated on the dried basis.

Description Fenoterol Hydrobromide is a white crystalline powder.
Fenoterol Hydrobromide is soluble in ethanol (95).

Identification (1) Dissolve 10 mg of Fenoterol Hydrobromide in 2 w/v % solution of sodium tetraborate decahydrate to make 50 mL. Add 1 mL of 1 w/v % solution of aminopyrazolone, 10 mL of 2 w/v % solution of potassium ferricyanide and 10 mL of dichloromethane, mix and allow to stand: a red-brown color develops in the lower layer.

(2) Prepare 0.037 w/v % solutions in diluted hydrochloric acid (1 in 10000) of Fenoterol Hydrobromide and Fenoterol Hydrobromide RS, respectively. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Fenoterol Hydrobromide and Fenoterol Hydrobromide RS as directed in potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Dissolve 10 mg each of Fenoterol Hydrobromide and Fenoterol Hydrobromide RS in ethanol (95) to make 10 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water and ammonia solution (28) (90 : 10 : 1.5) to a distance of about 15 cm and air-dry the plate. Spray evenly 1 w/v % potassium permanganate solution on the plate: the principal spot from the test solution shows the same R_f value as the principal spot from the standard solution.

(5) A solution of Fenoterol Hydrobromide (1 in 100) responds to the Qualitative Test (1) for bromides.

pH Dissolve 2.0 g of Fenoterol Hydrobromide in water to make exactly 50: the pH of this solution is between 4.2 and 5.2.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of Fenoterol Hydrobomide in water to make exactly 50 mL: the solution is clear and colorless.

(2) ***Iron***—Proceed with 1.0 g of Fenoterol Hydrobomide according to Method 3 and perform the test according to Method A. Prepare the control solution with 0.5 mL of standard iron solution (not more than 5 ppm).

(3) ***Phenone***—Dissolve 2.0 g of Fenoterol Hydrobomide in water to make exactly 50 mL and Determine the absorbance of this solution at 330 nm, as directed under Ultraviolet-visible Spectrophotometry: the absorbance is not more than 0.42 (not more than 0.2 %)

(4) ***Isomer***—Dissolve 20 mg each of Fenoterol Hydrobromide and Fenoterol Hydrobromide RS in water to make 10 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak heights, H_T and H_S of peak eluted right after the principal peak, for the test solution and the standard solution and calculate the amount of isomer: the amount of isomer is not more than 4.0 %.

$$\text{Amount(\%) of isomer} = \text{Amount(\%) of isomer labeled on Reference Standard} \times \frac{H_T}{H_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase : Mix 10 mL of 0.9 w/v % potassium dihydrogen phosphate and 690 mL of 2.5 w/v % sodim hydrogen phosphate dodecahydrate and add phosphoric acid to adjust pH to 8.5, add 300 mL of methanol and mix.

Flow rate: 1.0 mL/minute. Adjust the flow rate so that the retention time of the principal peak is not more than 20 minutes.

System suitability

System performance: When the procedure is run with 20 μL of this solution under the above operating conditions, adjust the sensitivity so that the height of peak eluted right after the principal peak is not less than 10 % of the full scale and the height of valley between the principal peak and the isomer peak is not more than 4 % of the full scale.

Loss on Drying Not more than 0.5 % (0.5 g, 105 ºC, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

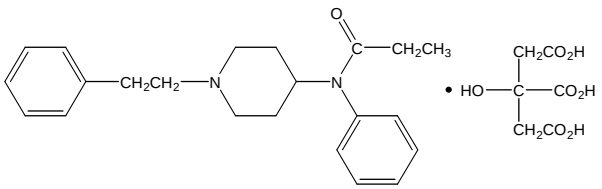
Assay Weigh accurately about 0.6 g of Fenoterol Hydrobromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid TS and 25 mL of 0.1 mol/L silver nitrate VS, shake and titrate with 0.1 mol/L ammonium thiocyanate VS until an orange color is obtained (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 38.48 mg of $C_{17}H_{21}NO_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Fentanyl Citrate



$C_{22}H_{28}N_2 \cdot OC_6H_8O_7$: 528.60

2-Hydroxypropane-1,2,3-tricarboxylic acid; *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide [*990-73-8*]

Fentanyl Citrate contains not less than 98.0 % and not less than 101.0 % of fentanyl citrate ($C_{22}H_{28}N_2 \cdot OC_6H_8O_7$), calculated on the dried basis.

Description Fentanyl Citrate appears as white crystals or crystalline powder.
Fentanyl Citrate is freely soluble in methanol or in acetic acid (100), sparingly soluble in water or in ethanol (95) and very slightly soluble in ether.

Identification (1) Dissolve 50 mg each of Fentanyl Citrate and Fentanyl Citrate RS in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Fentanyl Citrate and Fentanyl Citrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Fentanyl Citrate (1 in 100) responds to the Qualitative Tests (1) for citrate.

Melting Point 150 ~ 154 °C.

pH Dissolve 0.10 g of Fentanyl Citrate in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Fentanyl Citrate according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Fentanyl Citrate in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (31) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.2 g, in vacuum, silica gel, 60 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (0.5 g).

Assay Weigh accurately about 75 mg of Fentanyl Citrate, dissolve in 50 mL of acetic acid (100) and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

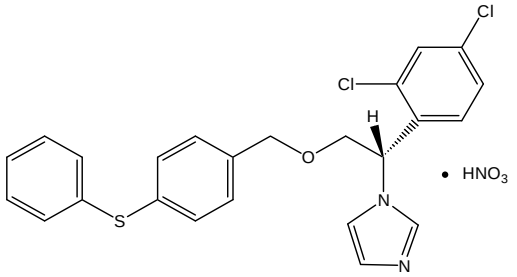
Each mL of 0.02 mol/L perchloric acid VS

= 10.572 mg of $C_{22}H_{28}N_2O \cdot C_6H_8O_7$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fenticonazole Nitrate



and enantiomer

$C_{24}H_{20}Cl_2N_2OS \cdot HNO_3$: 518.41

1-{2-(2,4-Dichlorophenyl)-2-[4-(phenylthio) enzyloxy]ethyl}-1*H*-imidazole nitrate [*73151-29-8*]

Fenticonazole Nitrate contains not less than 99.0 % and not more than 101.0 % of fenticonazole nitrate ($C_{24}H_{20}Cl_2N_2OS \cdot HNO_3$), calculated on the dried basis.

Description Fenticonazole Nitrate is a white powder.
Fenticonazole Nitrate is freely soluble in methanol or in *N,N*-dimethylformamide, sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Fenticonazole Nitrate and Fenticonazole Nitrate RS in ethanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Fenticonazole Nitrate and Fenticonazole Nitrate RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Weigh a portion of Fenticonazole Nitrate equivalent to about 1 mg of nitrate ion, mix with a mixture of 0.1 mL of nitrobenzene and 0.2 mL of sulfuric acid, stand for 5 minutes, cool in ice-water, add gently 5 mL of water while stirring. Add 5 mL of 10 mol/L sodium hydroxide TS and 5 mL of of aceton, shake and stand: a color of deep purple develops in the upper layer.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10° (0.1 g, methanol, 10 mL, 100 mm).

Meting Point 134 ~ 137 °C

Purity (1) ***Toluene***—Weigh exactly 0.2 g of

Fenticonazole Nitrate in a 10-mL vial, add exactly 5 mL of water to disperse and use this solution as the test solution, Separately, add 4.0 mg of toluene to water to make 1000 mL. Take 5 mL of this solution to a 10-mL vial and use it as the standard solution. Perform the test with the test solution and the standard solution as directed in the standard addition method under Gas Chromatography according to the following operating conditions. Use a head-space sample introduction device to measure the amount of toluene by the standard addition method: the amount of toluene is not more than 100 ppm.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 0.32 mm in internal diameter and about 25 m in length, coated with poly(cyanopropyl)(7)phenyl(7)methyl(86)siloxane for gas chromatography (1.2 μm in film thickness).

Headspace conditions: Equilibration temperature - 90 °C, equilibration time - 1 hour

Column temperature: 80 ºC.

Inlet temperature: 180 ºC.

Detector temperature: 220 ºC.

Carrier gas: Helium.

Split ratio: about 1 : 25

Column head pressure: 40 kPa.

Injection volume: Maintain each solution at 90 ºC for 1 hour and transfer 1 mL of the vapor phase onto the column.

(2) ***Related substances***—Dissolve about 25.0 mg of Fenticonazole Nitrate, accurately weighed, in the mobile phase to make exactly 25 mL and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add the mobile phase to make exactly 25 mL, and use this solution as the standard solution (2). Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (3). Pipet 5 mL of the test solution, add 5.0 mg of fenticonazole related substance I RS {(*RS*)-1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-3-[4-(phenylsulphanyl) benzyl]imidazolium nitrate} add the mobile phase to make exactly 100 mL, pipet 2 mL of this solution, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (4). Perform the test with 10 μL each of the test solution and the standard solution (1) as directed under Gas Chromatography according to the following operating conditions and determine the area of each peak by the automatic integration method: the area of any peak other than the principal peak and the nitrate ion peak (which correspond to the dead volume of the column) obtained from the test solution is not greater than the area of the principal peak obtained from the standard solution (2) (0.2 %) and the sum of the areas of such peaks is not greater than the area of the principal peak obtained from the standard solution (1) (0.5 %). Disregard any peak with an area less than that of the principal peak from the standard solution (3).

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 229 nm).

Column : A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase : A mixture of acetonitrilethe and phosphate buffer (70 : 30)

Flow rate: 1.0 mL/minute

System suitability

The procedure is run with 10 μL of the standard solution (2) under the above operating conditions, and adjust the sensitivity of the system so that the height of fenticonazole peak is not less than 10 % of the full scale of the recorder. When the procedure is run with 10 μL each of the standard solution (3) and the standard solution (4) under the above operating conditions, the resolution between the peaks of fenticonazole related substance I and fenticonazole is not less than 2, and the signal-to-noise ratio of the chromatogram obtained with the standard solution (3) is not less than 5.

Phosphate buffer—Dissolve 3.4 g of potassium dihydrogen phosphare in 900 mL of water, adjusting to pH 3.0 with phosphoric acid, and add water to make 1000 mL.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 °C, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

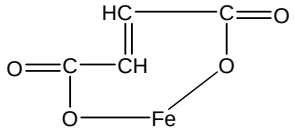
Assay Weigh accurately about 0.45 g of Fenticonazole Nitrate, dissolve in 50 mL of a mixture of 2-butanone and acetic acid (100) (1 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.84 mg of $C_{24}H_{20}Cl_2N_2OS \cdot HNO_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Ferrous Fumarate



$C_4H_2FeO_4$: 169.90

Iron (II) (*E*)-but-2-enedioate [*141-01-5*]

Ferrous Fumarate, when dried, contains not less than 97.0 % and not more than 101.0 % of ferrous fumarate ($C_4H_2FeO_4$).

Description Ferrous Fumarate appears as orange to red-brown powder and is odorless.
Ferrous Fumarate is slightly soluble in water and very slightly soluble in ethanol (95).
The solubility of Ferrous Fumarate in dilute hydrochloric acid is decreased by liberation of fumaric acid.

Identification (1) To 1.5 g of Ferrous Fumarate, add 25 mL of diluted hydrochloric acid (1 in 2), dilute with water to make 50 mL, heat to obtain complete solution, then cool, filter on a glass filter, wash the precipitate with diluted hydrochloric acid (3 in 100) and dry the precipitate at 105 °C. Determine the infrared spectra of the dried precipitate and Fumaric Acid RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The filtrate of (1) responds to the Qualitative Tests for ferrous salt.

Purity (1) ***Sulfate***—Transfer 1.0 g of Ferrous Fumarate to a beaker, add 100 mL of water and heat on a water-bath, adding hydrochloric acid drop-wise until complete solution is obtained (about 2 mL of the acid is required). Filter the solution, if necessary, and dilute the filtrate with water to make 100 mL. Heat this solution to boiling, add 10 mL of barium chloride TS, warm on a water-bath for 2 hours, cover and allow to stand for 16 hours. If crystals of ferrous fumarate form, warm the solution on a water-bath to dissolve them. Filter the solution through weighing filter paper, add ammonium sulfide TS to the filtrate, wash the residue with hot water until no more black precipitate is produced and transfer the paper containing the residue to a previously tared crucible. Char the paper, without burning and ignite the crucible and its contents at 600 °C to a constant mass: each mg of residue is equivalent to 0.412 mg of sulfate (SO_4) (not more than 0.2 %).

(2) ***Arsenic***—Transfer about 2.0 g of Ferrous Fumarate to a beaker and add 10 mL of water and 10 mL of sulfuric acid. Warm to precipitate fumaric acid completely, cool, add 20 mL of water and filter into a 50 mL volumetric flask. Wash the precipitate with water, add the washings to the flask, add water to to the mark. Perform the test with 25 mL of this solution. Prepare the control solution with 3.0 mL of standard arsenic solution (not more than 3 ppm).

(3) ***Ferric ion***—Transfer about 2.0 g of Ferrous Fumarate, accurately weighed, to a glass-stoppered, Erlenmyer flask, add 25 mL of water and 4 mL of hydrochloric acid and heat on a hot plate until complete solution is obtained. Stopper the flask and cool to room temperature. Add 3 g of potassium iodide, stopper the flask, swirl to mix and allow to stand in the dark for 5 minutes. Remove the stopper, add 75 mL of water and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Not more than 7.16 mL of 0.1 mol/L sodium thiosulfate is consumed (not more than 2.0 %).

(4) ***Mercury***—Perform the test with light-resistant container. Dissolve about 1 g of Ferrous Fumarate, accurately weighed, in 30 mL of diluted nitric acid (1 in 10), by heating on a water-bath. Cool quickly by immersion in an ice-bath and filter through a filter (G4), previously washed with diluted nitric acid (1 in 10) and water. To the filtrate, add 20 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylamine hydrochloride TS and use this solution as the test solution. Separately, prepare a control solution consisting of 3.0 mL of standard mercury solution, 30 mL of diluted nitric acid (1 in 10), 5 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylamine hydrochloride TS. To the control solution, add ammonium hydroxide TS to adjust to a pH of 1.8, add sulfuric acid to the test solution to adjust to a pH of 1.8 and transfer to a separator, respectively. Perform the test with the test solution and the control solution as follows. Extract with two 5 mL volumes of dithizone solution for extraction and 5 mL of chloroform and transfer the chloroform extracts to a second separator. Add 10 mL of diluted hydrochloric acid (1 in 2), shake to mix, allow the layers to separate and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform and discard the washing. Add 0.1 mL of disodium ethylenediamine tetraacetate solution (1 in 50) and 2 mL of 6 mol/L acetic acid, mix and add slowly 5 mL of ammonia TS. Stopper the separator and cool under cold running water. Remove the stopper and pour the contents into a beaker. Adjust the test solution and the control solution to a pH of 1.8 in the same manner as before and return the solution to its separator, respectively. Add 5.0 mL of diluted dithizone solution for extraction, shake vigorously and allow the layers to separate. At this point, compare the colors observed in the chloroform layers of the two solutions that have been treated in parallel: the color observed by the test solution is not more intense than that observed by the control solution (not more than 3 ppm).

Mercury stock solution—Transfer 135.4 mg of mercury (II) chloride to a volumetric flask, dissolve in 0.5 mol/L sulfuric acid and add 0.5 mol/L sulfuric acid to make 100 mL and mix. This solution contains 0.1 g of mercury (Hg) in 100 mL.

Mercury standard solution—Transfer 1.0 mL of mercury stock solution to a volumetric flask before use and add 0.5 mol/L sulfuric acid to make 1000 mL and mix. This solution contains 1 g of mercury (Hg) in 1 mL.

Diluted dithizone solution for extraction—Dilute 5 mL of dithizone solution for extraction with 25 mL of chloroform before use.

(5) ***Lead***—Add 1.0 g of Ferrous Fumarate to a beaker, add 6 mL of nitric acid and 10 mL of perchloric acid, cover with a watch glass and heat until complete dryness. Cool, dissolve the residue in 10 mL of 9 mol/L hydrochloric acid and transfer with the aid of about 10 mL of water to a volumetric flask. Add 20 mL of ascorbic acid-sodium iodide solution and 5.0 mL of trioctylphosphine oxide solution, shake for 30 seconds and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again and allow to separate. Use the organic solvent layer as the test solution. Separately, transfer 5.0 mL of lead stock solution and add water to make exactly 100 mL. Transfer exactly 2 mL of the resulting solution to a beaker. To this beaker and to a second empty beaker, add 6 mL of nitric acid and 10 mL of perchloric acid and evaporate to dryness. Cool, dissolve the residue in 10 mL of 9 mol/L hydrochloric acid and transfer with the aid of about 10 mL of water to a volumetric flask. To each flask, add 20 mL of ascorbic acid-sodium iodide solution and 5.0 mL of trioctylphosphine oxide solution, shake for 30 seconds and allow to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again and allow to separate. Use the organic solvent layers as the standard solution (2.0 μg/mL) and the blank solution, respectively. Determine the absorbances of the blank solution, the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometer according to the following conditions: the absorbance of the test solution doses not exceed that of the standard solution (not more than 0.001 %). But, using 4-methyl-2-pentanone to set the instrument to zero, the absorbance of the blank solution is not greater than 20 % of the difference between the absorbance of the standard preparation and that of the blank solution.

Gas used: Dissolved acetylene – Air
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

Trioctylphosphine oxide solution—Dissolve 5.0 g of trioctylphosphine oxide in 4-methyl-2-pentanone to make 100 mL.

(6) ***Cadmium***—Dissolve 2.0 g of Ferrous Fumarate in 10 mL of hydrochloric acid and 80 mL of water, and heat gently if necessary. After cooling, filter if necessary, add water to make 100 mL, and use this solution as the test solution. Separately, pipet 2.0 mL of standard cadmium solution, add hydrochloric acid to make exactly 10 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Atomic Absorption Spectrophotometry: the absorbance of the test solution is not more than that of the standard solution (not more than 10 ppm).

Gas used: Dissolved acetylene – Air
Lamp: Cadmium hollow cathode lamp
Wavelength: 228.8 nm

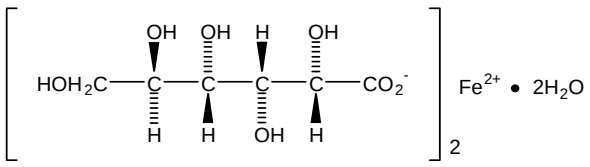
Loss on Drying Not more than 1.5 % (1 g, 105 °C, 16 hours).

Assay Transfer about 0.5 g of Ferrous Fumarate, accurately weighed, to an Erlenmyer flask, add 25 mL of diluted hydrochloric acid (2 in 5) and heat to boiling. Add a solution of 5.6 g of tin (II) chloride dihydrate in 50 mL of diluted hydrochloric acid (3 in 10) until yellow color disappears, then add 2 drops in excess. Cool the solution in an ice-bath to room temperature, add 10 mL of mercury (II) chloride solution (1 in 20) and allow to stand for 5 minutes. Add 200 mL of water, 25 mL of diluted sulfuric acid (1 in 2) and 4 mL of phosphoric acid and then titrate with 0.1 mol/L ceric ammonium sulfate VS (indicator: 2 drops of 1,10-phenanthroline monohydrate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L ceric ammonium sulfate VS
= 16.990 mg of $C_4H_2FeO_4$

Containers and Storage ***Containers***—Well-closed containers.

Ferrous Gluconate Hydrate



$C_{12}H_{22}FeO_{14}\cdot 2H_2O$: 482.17

Iron(2+);(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanoate; dihydrate [*6047-12-7*]

Ferrous Gluconate Hydrate, when dried, contains not less than 97.0 % and not more than 102.0 % of ferrous gluconate ($C_{12}H_{22}FeO_{14}$: 446.14).

Description Ferrous Gluconate Hydrate is a yellowish gray or pale yellow green fine powder or granule and has a slight odor like sulfur-burning.
One gram of Ferrous Gluconate Hydrate is soluble in 10 mL of hot water and very slightly soluble in ethanol

(95).
An aqueous solution of ferrous gluconate (1 in 20) is acidic.

Identification (1) Dissolve about 10 mg of Ferrous Gluconate Hydrate in 10 mL of water, if necessary, heat on a water-bath at 60 ºC and use this solution as the test solution. Separately, dissolve 10 mg of Ferrous Gluconate Hydrate RS in 10 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as diredted under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on the plate of silica gel for thin-layer chromatographic. Develop the plate with of a mixture of ethanol (95), water, ammonia solution (28) and acetic acid (50 : 30 : 10 : 10) to a distance of about 15 cm and dry at 110 ºC for 20 minutes. Allow to cool, spray with a spray reagent prepared as follow; dissolve 2.5 g of ammonium molybdate tetrahydrate in about 50 mL of 1 mol/L sulfuric acid TS in a volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, add 1 mol/L sulfuric acid TS to make 100 mL and mix. Heat the plate at 110 ºC for about 10 minutes: the color and R_f value of the principal spot obtained from the test solution should be the same as those obtained from the standard solution.

(2) Addition of potassium hexacyanoferrate (III) TS to an aqueous solution of Ferrous Gluconate Hydrate (1 in 200): dark blue precipitation is produced.

Purity (1) ***Chloride***—Proceed with 1.0 g of Ferrouse Gluconate Hydrate. Prepare the control solution with 1.0 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.07 %).

(2) ***Sulfate***—Proceed with 1.0 g of Ferrous Gluconate Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L sulfuric acid VS (not more than 0.1 %).

(3) ***Oxalic acid***—Dissolve 1.0 g of Ferrous Gluconate Hydrate in 10 mL of water, add 2 mL of hydrochloric acid and transfer to a separator. Extract successively with 50 mL and 20 mL volumes of ether. Combine the ether extracts, add 10 mL of water and evaporate the ether on a steam-bath. Add 1 drop of 6 mol/L acetic acid and 1 mL of calcium acetate hydrate solution (1 in 20): no turbidity is produced within 5 minutes.

(4) ***Lead***—Add 1.0 g of Ferrous Gluconate Hydrate, 10 mL of 9 mol/L hydrochloric acid TS, about 10 mL of water, 20 mL of ascorbic acid, sodium iodide solution and 5.0 mL of triocytylphosphine oxide in 4-methyl-2-pentanone solution (5 in 100) to a volumetric flask, shake for 30 seconds and allow to separate. Add water into the neck of the flask, shake again and allow to separate. The organic solvent layer collected is used as the test solution. Separately, pipet 5.0 mL of lead nitrate standard stock solution into a volumetric flask, dilute with water to make 100 mL, pipet 2.0 mL of the solution to a volumetric flask and then prepare the standard solution, as directed under the test solution. Determine the absorbances of the test solution and the standard solution according to the following conditions, as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the solution obtained from the test solution is not greater than that from the standard solution (not more than 0.001 %).

Gas: Dissolved acetylene – Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.8 nm.

(5) ***Mercury***—Perform the test using light-resistant vessels. Weigh accurately about 1 g of Ferrous Gluconate Hydrate, add 30 mL of diluted nitric acid (1 in 10) and dissolve by heating on a water-bath. Quickly transfer to an ice bath, allow to cool and filter through a filter washed with water, using previously diluted nitric acid (1 in 10). To the filtrate, add 20 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylammonium chloride, and use this solution as the test solution. Separately, prepare a control solution with 3.0 mL of standard mercury solution, 30 mL of diluted nitric acid (1 in 4), 5 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylammonium chloride. Transfer each of the test solution and the control solution to a separatory funnel, adjust to pH 1.8 with sulfuric acid, extract with two 5 mL volumes of dithizone solution for extraction and 5 mL volumes of chloroform, and transfer the chloroform extract to a different separatory funnel. Add 10 mL of diluted hydrochloric acid (1 in 2), shake, allow to stand and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform and discard the washing. Add 0.1 mL of ethylenediamine tetraacetic acid disodium solution (1 in 50) and 2 mL of 6 mol/L acetic acid, mix and slowly add 5 mL of ammonia TS. Stopper the separator and cool under cold running water. Remove the stopper and pour the contents into a beaker. Adjust the test solution and the control solution to pH 1.8 in the same manner as above and transfer each to a separatory funnel. Add 5.0 mL of diluted dithizone solution for extraction, shake vigorously and allow to stand. Compare the color that appears in the chloroform layer of the test solution and the control solution, using the diluted dithizone solution for extraction as the blank: the color of the test solution is not more intense than that of the control solution (not more than 3 ppm).

Mercury stock solution—Transfer 135.4 mg of mercury (II) chloride to a volumetric flask, dissolve in 0.5 mol/L sulfuric acid TS and add 0.5 mol/L sulfuric acid TS to make 100 mL. This solution contains 0.1 g of mercury (Hg) in 100 mL.

Mercury standard solution—Transfer 1.0 mL of the mercury stock solution to a volumetric flask before use, add 0.5 mol/L sulfuric acid TS to make 1000 mL, and mix. This solution contains 1 μg of mercury (Hg) in 1 mL.

Diluted dithizone solution for extraction—Dilute 5 mL of dithizone solution for extraction with 25 mL of chloroform before use.

(6) ***Ferric ion***—Dissolve about 5 g of Ferrous Gluconate Hydrate, exactly weighed, in a mixture of 100 mL of water and 10 mL of hydrochloric acid and add 3 g of potassium iodide. Shake and allow to stand in the dark place for 5 minutes. Titrate any liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination and make any necessary correction (not more than 2.0 %).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 5.585 mg of Fe^{+3}

(7) ***Arsenic***—Weigh 1.0 g of Ferrous Gluconate Hydrate in a round-bottom flask and add 40 mL of 4.5 mol/L sulfuric acid and 2 mL of potassium bromide solution (3 in 10). Immediately collect to a suitable distillation apparatus having a reservoir with a water jacket, cooled with circulating ice–water and heat to dissolve the test specimen. Prepare 25 mL of distillate as the test solution and perform the test (not more than 3 ppm).

(8) ***Reducing sugars***—Dissolve about 500 mg of Ferrous Gluconate Hydrate in 10 mL of water, warm and render alkaline with 1 mL of 6 mol/L ammonium hydroxide TS. Pass hydrogen sulfide gas into the solution to precipitate the iron and allow to stand for 30 minutes. Filter and wash the precipitate with two successive 5 mL volumes of water. Acidify the combined filtrate and washings with hydrochloric acid and add 2 mL of dilute hydrochloric acid. Boil the solution until the vapors no longer darken lead acetate paper and continue to boil, if necessary, until concentrated to be about 10 mL. Cool, add 5 mL of sodium carbonate TS and 20 mL of water, filter and wash the residue with water. Combine the filtrate and washing and add water to make exactly 100 mL. Take 5 mL of this solution, add 2 mL of Fehling TS and boil for 1 minute: no red precipitate is produced within 1 minute.

Loss on Drying 6.5 ~ 10.0 % (1 g, 105 ºC, 16 hours).

Assay Dissolve about 1.5 g of Ferrous Gluconate Hydrate, accurately weighed, in a mixture of 75 mL of water and 15 mL of dilute sulfuric acid in a cornical-flask. Add 250 mg of zinc powder, close the flask with a stopper containing a Bunsen valve and allow to stand at room temperature for 20 minutes or until the solution becomes colorless. Filter the solution through a filtering crucible containing an asbestos mat coated with a thin-layer of zinc dust and wash the crucible and contents with 10 mL of dilute sulfuric acid, followed by 10 mL of water. Add 1,10-phenanthroline monohydrate TS and titrate the filtrate in the suction flask immediately with 0.1 mol/L cerium sulfate VS. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L cerium sulfate VS
= 44.61 mg of $C_{12}H_{22}FeO_{14}$

Containers and Storage ***Containers***—Tight containers.

Ferrous Gluconate Tablets

$C_{12}H_{22}FeO_{14}\cdot 2\ H_2O$: 482.17

Ferrous Gluconate Tablets contain not less than 93.0 % and not more than 107.0 % of ferrous gluconate hydrate ($C_{12}H_{22}FeO_{14}\cdot 2\ H_2O$: 482.17).

Method of Preparation Prepare as directed under Tablets, with Ferrous Gluconate Hydrate.

Identification Take a portion of powdered Ferrous Gluconate Tablets, equivalent to about 1 g of Ferrous Gluconate Hydrate according to the labeled amount, add 100 mL of water and filter: the suitable volume of the filtrate is diluted with water, to a designated concentration. Perform the test with the solution as directed in the Identification under Ferrous Gluconate Hydrate.

Dissolution Test Perform the test with 1 tablet of Ferrous Gluconate Tablets at 150 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of the 1st solution as the dissolution solution. Take the dissolved solution 80 minutes after the start of the test, filter through a membrane filter and use the filtrate solution as the test solution. If necessary, dilute the test solution as a suitable concentration using the test solution. Separately, weigh accurately a portion of Ferrous Gluconate Hydrate RS, add the test solution to dissolve and prepare the standard solution containing the same iron concentration as the test solution. Determine the absorbances of the test solution and the standard solution according to the following conditions, as directed under the Atomic Absorption Spectrophotometry. Calculate the content of iron using the calibration curve obtained from the absorbance of standard solution.

Gas used: Dissolved acetylene - Air
Lamp: Iron hollow-cathode lamp.
Wavelength: 248.3 nm

The dissolution rate of Ferrous Gluconate Tablets in 80 minutes is not less than 80.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh and finely powder not less than 20 Ferrous Gluconate Tablets. Weigh accurately a portion of the powder, equivalent to about 1.5 g of Ferrous Gluconate Hydrate and dissolve in a mixture of 75 mL

of water and 15 mL of dilute sulfuric acid in a cornical-flask. Proceed as directed in the Assay under Ferrous Gluconate Hydrate.

Each mL of 0.1 mol/L ammonium ceric sulfate VS
= 48.22 mg of $C_{12}H_{22}FeO_{14}.2H_2O$

Containers and Storage ***Containers***—Tight containers.

Ferrous Sulfate Hydrate

Ferrous Sulfate $FeSO_4 \cdot 7H_2O$: 278.02

Iron(2+) sulfate heptahydrate [*7782-63-0*]

Ferrous Sulfate Hydrate contains not less than 98.0 % and not more than 104.0 % of ferrous sulfate ($FeSO_4 \cdot 7H_2O$).

Description Ferrous Sulfate Hydrate appears as pale green crystals or crystalline powder, is odorless and has an astringent taste.
Ferrous Sulfate Hydrate is freely soluble in water and practically insoluble in ethanol (95).
Ferrous Sulfate Hydrate is efflorescent in dry air and its crytalline surface becomes yellow-brown in moist air.

Identification A solution of Ferrous Sulfate Hydrate (1 in 10) responds to the Qualitative Tests for ferrous salt and for sulfate.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Ferrous Sulfate Hydrate in 20 mL of water and 1 mL of dilute sulfuric acid: the solution is clear.

(2) ***Acid***—Take 5.0 g of powdered Ferrous Sulfate Hydrate, add 50 mL of ethanol (95), shake well for 2 minutes and filter the mixture. To 25 mL of the filtrate, add 50 mL of water, 3 drops of bromothymol blue TS and 0.5 mL of dilute sodium hydroxide TS: a blue color is observed.

(3) ***Mercury***—Perform the test with light-resistant container. Dissolve about 1 g of Ferrous Sulfate Hydrate, accurately weighed, in 30 mL of diluted nitric acid (1 in 10), by heating on a water-bath. Cool quickly by immersion in an ice-bath and filter through a filter (G4), previously washed with diluted nitric acid (1 in 10) and water. To the filtrate, add 20 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylamine hydrochloride TS and use this solution as the test solution. Separately, prepare a control solution consisting of 3.0 mL of standard mercury solution, 30 mL of diluted nitric acid (1 in 10), 5 mL of sodium citrate solution (1 in 4) and 1 mL of hydroxylamine hydrochloride TS. To the control solution, add ammonium hydroxide TS to adjust to a pH of 1.8, add sulfuric acid to the test solution to adjust to a pH of 1.8 and transfer to a separator, respectively. Perform the test with the test solution and the control solution as follows. Extract with two 5 mL volumes of dithizone solution for extraction and 5 mL of chloroform and transfer the chloroform extracts to a second separator. Add 10 mL of diluted hydrochloric acid (1 in 2), shake to mix, allow the layers to separate and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform and discard the washing. Add 0.1 mL of disodium ethylenediamine tetraacetate solution (1 in 50) and 2 mL of 6 mol/L acetic acid, mix and add slowly 5 mL of ammonia TS. Stopper the separator and cool under cold running water. Remove the stopper and pour the contents into a beaker. Adjust the test solution and the control solution to a pH of 1.8 in the same manner as before and return the solution to its separator, respectively. Add 5.0 mL of diluted dithizone solution for extraction, shake vigorously and allow the layers to separate. At this point, compare the colors observed in the chloroform layers of the two solutions that have been treated in parallel: the color observed by the test solution is not more intense than that observed by the control solution (not more than 3 ppm).

Mercury stock solution—Transfer 135.4 mg of mercury (II) chloride to a volumetric flask, dissolve in 0.5 mol/L sulfuric acid and add 0.5 mol/L sulfuric acid to make 100 mL and mix. This solution contains 0.1 g of mercury (Hg) in 100 mL.

Mercury standard solution—Transfer 1.0 mL of mercury stock solution to a volumetric flask before use and add 0.5 mol/L sulfuric acid to make 1000 mL and mix. This solution contains 1 g of mercury (Hg) in 1 mL.

Diluted dithizone solution for extraction—Dilute 5 mL of dithizone solution for extraction with 25 mL of chloroform before use.

(4) ***Lead***—Take 1.0 g of Ferrous Sulfate Hydrate in a 50 mL volumetric beaker, add 10 mL of 9 mol/L hydrochloric acid about 10 mL of water, 20 mL of ascorbic acid-sodium iodide solution and 5 mL of trioctylphosphine oxide solution in 4-methyl-2-pentanone (5 in 100), shake for 30 seconds and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again and allow to separate. Use the organic solvent layer as the test solution. Separately, transfer exactly 5 mL of lead stock solution and add water to make exactly 100 mL. Transfer exactly 2 mL of the resulting solution to a 50 mL volumetric flask and proceed as the test solution and use this soluteon as the standard solution. Determine the absorbances of the the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometer according to the following conditions: the absorbance of the test solution doses not exceed that of the standard solution.

Gas used: Dissolved acetylene – Air
Lamp: A lead hollow-cathode lamp.

Wavelength: 283.3 nm.

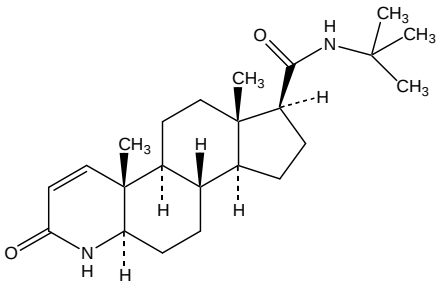
(5) ***Arsenic***—Prepare the control solution with 1.0 g of Ferrous Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.7 g of Ferrous Sulfate Hydrate, add 20 mL of water, 20 mL of dilute sulfuric acid TS and 2 mL of phosphoric acid, and immediately titrate with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
= 27.80 mg of $FeSO_4 \cdot 7H_2O$

Containers and Storage ***Containers***—Tight containers.

Finasteride



$C_{23}H_{36}N_2O_2$: 372.54

(1*S*,3a*S*,3b*S*,5a*R*,9a*R*,9b*S*,11a*S*)-*N*-*tert*-Butyl-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide [*98319-26-7*]

Finasteride contains not less than 98.5 % and not more than 101.0 % of finasterid ($C_{23}H_{36}N_2O_2$), calculated on the anhydrous basis.

Description Finasteride appear as white or grayish white crystalline powder.
Finateride is freely soluble in ethanol (95), or in chloroform, and very slightly soluble in water.
Melting point—about 257 ºC

Identification (1) Determine the infrared spectra of Finateride and Finasteride RS, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak obtained from the test solution under Assay is equal to that of the principal peak obtained from the standard solution.

Specific Optical Rotation $[\alpha]_{405nm}^{25}$: -56.0 ~ -60.0°
(0.1 g, methanol, 10 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Finasteride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve about 0.1 g of Finasteride, accurately weighed, in a mixture of water and acetonitrile (1 : 1) to make exactly 100 mL and use this solution as the test solution. Perform the test with 15 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of each peak by the automatic integration method and calculate the amount of each related substance: the amount of any related subtances is not more than 0.5 % and the total amount of related substances is not more than 1.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_s}$$

A_i: Peak area of each related substance
A_s: Sum of all peak area obtained from the test solution

Operating conditions
Detector : An ultraviolet absorption photometer (wavelength: 210 nm).
Column : A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).
Mobile phase : A mixture of water, tetrahydrofuran and acetonitrile (8 : 1 : 1)
Column temperature: A constant temperature of about 60 °C
Flow rate: 1.5 mL/minute
System suitability
System performance: Dissolve 10 mg of Finasteride RS in a mixture of water and acetonitrile (1 : 1) to make 10 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of finasteride are not less than 10000 and not more than 1.3, respectively.

Water Not more than 0.3 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve about 20 mg each of Finateride and Finasteride RS, accurately weighed, in a mixture of water and acetonitrile (1 : 1) to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of the principal peak

for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of finasteride } (C_{23}H_{36}N_2O_2) = \text{Amount (mg) of Finasteride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 215 nm).

Column : A stainless steel column, about 3.0 mm in internal diameter and about 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase : A mixture of water and tetrahydrofuran (4 : 1)

Flow rate: about 3 mL/minute

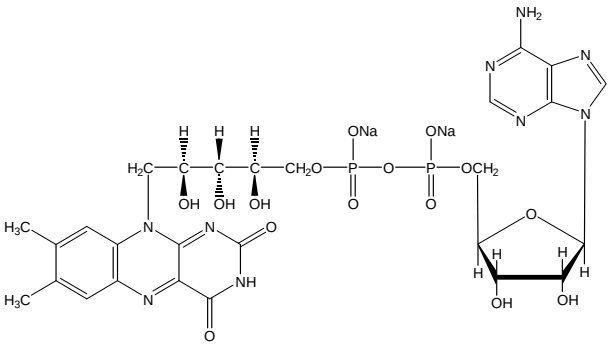
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of finasteride are not less than 1800 and not more than 1.3, respectively.

System reproducibility: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative deviation of the peak area is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Flavin Adenine Dinucleotide Sodium



$C_{27}H_{31}N_9Na_2O_{15}P_2$: 829.51

Disodium adenosine 5'-(3-{D-ribo-5-[7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2*H*)-yl]-2,3,4-trihydroxypentyl} dihydrogen diphosphate) [*84366-81-4*]

Flavin Adenine Dinucleotide Sodium contains not less than 93.0 % and not more than 101.0 % of flavin adenine dinucleotide sodium ($C_{27}H_{31}N_9Na_2O_{15}P_2$), calculated on the anhydrous basis.

Description Flavin Adenine Dinucleotide Sodium is an orange-yellow to pale yellow-brown powder, is odorless or has a slight, characteristic odor and has a slightly bitter taste.

Flavin Adenine Dinucleotide Sodium is freely soluble in water and practically insoluble in methanol, in ethanol (95), in ethylene glycol or in ether.

Flavin Adenine Dinucleotide Sodium is hygroscopic.

Flavin Adenine Dinucleotide Sodium is decomposed by light.

Identification (1) A solution of Flavin Adenine Dinucleotide Sodium (1 in 100000) is pale yellow-green in color and shows a strong yellow-green fluorescence. Take 5 mL of the solution, and add 20 mg of hydrosulfite sodium: the color and the fluorescence of the solution disappear and gradually reappear when the solution is shaken in air. Add dilute hydrochloric acid or sodium hydroxide TS drop-wise: the fluorescence of the solution disappears.

(2) Determine the infrared spectrum of Flavin Adenine Dinucleotide Sodium and Flavin Adenine Dinucleotide Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Take 0.1 g of Flavin Adenine Dinucleotide Sodium, add 10 mL of nitric acid, evaporate on a water-bath to dryness and ignite. To the residue, add 10 mL of diluted nitric acid (1 in 50), boil for 5 minutes and after cooling, neutralize with ammonia TS, then filter the solution, if necessary: the solution responds to the Qualitative Tests for sodium salt and the Qualitative Tests (1) and (3) for phosphate.

Specific Optical Rotation $[\alpha]_D^{20}$: -21.0 ~ -25.5° (0.3 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH Dissolve 1.0 g of Flavin Adenine Dinucleotide Sodium in 100 mL of water: the pH of this solution is between 5.5 and 6.5

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Flavin Adenine Dinucleotide Sodium in 10 mL of water: the solution is clear and orange-yellow in color.

(2) ***Free phosphoric acid***—Weigh accurately about 20 mg of Flavin Adenine Dinucleotide Sodium, dissolve in 10 mL of water and use this solution as the test solution. Separately, measure exactly 2 mL of standard phosphoric acid solution, add 10 mL of water and use this solution as the standard solution. To each of the test solution and the standard solution, add 2 mL of diluted perchloric acid (100 in 117), then add 1 mL of ammonium molybdate TS and 2 ml of 2,4-diaminophenol hydrochloride TS, respectively, shake, add water to make exactly 25 mL and allow to stand at 20 ± 1 ºC for 30 minutes. Perform the test with the test solution and the standard solution as directed under

Ultraviolet-visible Spectrophotometry, using a solution prepared in the same manner with 2 mL of water, as the blank and determine the absorbances, A_T and A_S, of the test solution and the standard solution at 730 nm, respectively: the amount of free phosphoric acid is less than 0.25 %.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 5.16$$

W: Amount (mg) of Flavin Adenine Dinucleotide Sodium, calculated on the anhydrous basis.

(3) ***Heavy metals***—Proceed with 1.0 g of Flavin Adenine Dinucleotide Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution With 2.0 g of Flavin Adenine Dinucleotide Sodium according to Method 3 and perform the test (not more than 1 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Flavin Adenine Dinucleotide Sodium in 200 mL of the mobile phase and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area, *A*, of Flavin Adenine Dinucleotide and the area, *S*, of peaks other than the peak of Flavin Adenine Dinucleotide by the automatic integration method: *S/(A+S)* is not more than 0.10.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column, column temperature, mobile phase, flow rate, selection of column: Proceed as directed in the operating conditions in the Procedure (ii) in the Assay (1).

System suitability

System performance: Proceed as directed in the system suitability in the Procedure (ii) in the Assay (1).

Test for required detection: To exactly 2 mL of the test solution, add the mobile phase to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of flavin adenine dinucleotide obtained from 20 μL of this solution is equivalent to 8 to 12 % of that of flavin adenine dinucleotide obtained from 20 μL of the test solution.

System repeatability: When the test is repeated 6 times with 20 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0 %.

Water Take 50 mL of a mixture of methanol for water determination and ethylene glycol for water determination (1 : 1) into a dry titration flask and titrate with Karl Fischer TS water determination until end point. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, transfer quickly to the titration flask, add an excess and constant volume of Karl Fischer TS water determination, dissolve by stirring for 10 minutes and perform the test: the water content is not more than 10.0 %.

Assay (1) ***Procedure***—(i) Total Flavin content:Perform this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium and dissolve in water to make exactly 200 mL. Pipet 5.0 mL of this solution, add 5 mL of zinc chloride TS and heat on a water-bath for 30 minutes. After cooling, add water to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105 ºC for 2 hours, dissolve in 200 mL of diluted acetic acid (100) (1 in 100) by warming, cool and add water to make exactly 500 mL. Measure exactly 10 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 450 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry, using water as the blank.

$$\text{Total amount (mg) of Flavin} = \text{Amount (mg) of Riboflavin RS} \times \frac{A_T}{A_S} \times \frac{4}{5}$$

(ii) Peak area ratio of Flavin Adenine Dinucleotide: Dissolve 0.1 g of Flavin Adenine Dinucleotide Sodium in 200 mL of water and use this solution as the test solution. Perform the test with 5 μL of this solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area, *A*, of flavin adenine dinucleotide and the total area, *S*, of the peaks other than flavin adenine dinucleotide by the automatic integration method.

$$\text{Peak area ratio of flavin adenine dinucleotide} = 1.08 \times \frac{1.08 \times A}{10.8 \times A + S}$$

Operating conditions

Detector: A visible spectrophotometer (wavelength: 450 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 35 ºC.

Mobile phase: A mixture of a solution of monobasic potassium phosphate (1 in 500) and methanol (4 : 1).

Flow rate: Adjust the flow rate so that the retention time of Flavin Adenine Dinucleotide is about 10 minutes.

System suitability

Test for required detectability: To exactly 2 mL of the test solution, add water to make exactly 20 mL, and use this solution as the system suitability solution. Pipet 2 mL of the solution, and add water to make exactly 20 mL. Confirm that the peak area of flavin adenine dinucleotide obtained from 5 μL of this solution is equivalent to 8 to 12 % of that of flavin adenine dinucleotide obtained from 5 μL of the system suitability solution

System performance: Dissolve about 20 mg each of Flavin Adenine Dinucleotide Sodium and riboflavin sodium phosphate in 100 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions and calculate the resolution, flavin adenine dinucleotide and riboflavin phosphate in this order are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatibility: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0 %.

Time span of measurement: About 4.5 times as long as the retention time of flavin adenine dinucleotide.

(2) ***Calculation***

Amount (mg) of flavin adenine dinucleotide sodium ($C_{27}H_{31}N_9Na_2O_{15}P_2$) = $f_T \times f_R \times 2.2040$

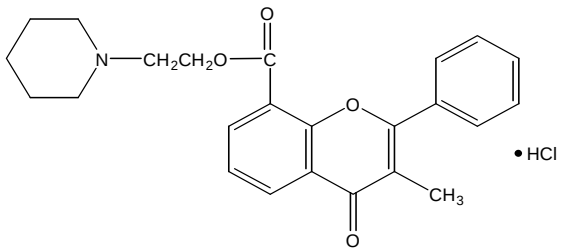
f_T: Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i).

f_R: Peak area ratio of flavin adenine dinucleotide in Flavin Adenine Dinucleotide Sodium obtained from the procedure (ii).

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Flavoxate Hydrochloride



$C_{24}H_{25}NO_4 \cdot HCl$: 427.92

2-(1-Piperidyl)ethyl 3-methyl-4-oxo-2-phenylchromene-8-carboxylate hydrochloride
[*3717-88-2*]

Flavoxate Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of flavoxate hydrochloried ($C_{24}H_{25}NO_4 \cdot HCl$).

Description Flavoxate Hydrochloride appears as white crystals or crystalline powder.

Flavoxate Hydrochloride is sparingly soluble in acetic acid (100) or in chloroform, slightly soluble in water or in ethanol (95) and practically insoluble in acetonitrile or in ether.

Identification (1) Determine the absorption spectra of the solutions of Flavoxate Hydrochloride and Flavoxate Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Flavoxate Hydrochloride and Flavoxate Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Flavoxate Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Flavoxate Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 2.0 g of Flavoxate Hydrochloride according to Method 4 and perform the test (not more than 1 ppm).

(3) ***Related substances***—Dissolve 80 mg of Flavoxate Hydrochloride in 10 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution and add chloroform to make exactly 20 mL, then pipet 1.0 mL of this solution, add chloroform to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, in vaccum, silica gel, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

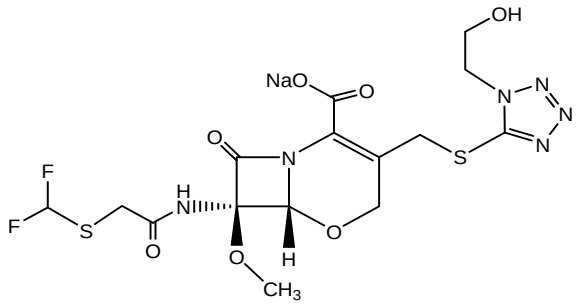
Assay Weigh accurately about 0.6 g of Flavoxate Hydrochloride, previously dried, add 10 mL of acetic acid (100) and 40 mL of acetonitrile to dissolve, add 50

mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.79 mg of $C_{24}H_{25}NO_4.HCl$

Containers and Storage ***Containers***—Tight containers.

Flomoxef Sodium



$C_{15}H_{17}F_2N_6NaO_7S_2$: 518.45

Sodium (6*R*,7*R*)-7-[[2-(difluoromethylsulfanyl)acetyl]amino]-3-[[1-(2-hydroxyethyl)tetrazol-5-yl]sulfanylmethyl]-7-methoxy-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [*92823-03-5*]

Flomoxef Sodium contains not less than 870 μg (potency) and not more than 985 μg (potency) per mg of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47), calculated on the anhydrous basis.

Description Flomoxef Sodium appears as white to pale yellow-white powder or masses.
Flomoxef Sodium is very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (99.5).

Identification (1) Proceed with 0.01 g of Flomoxef Sodium as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. To 2 mL of this test solution add 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3), and cerium (III) nitrate TS (1 : 1 : 1): a blue-purple color develops.

(2) Determine the absorption spectra of solutions of Flomoxef Sodium and Flomoxef Sodium RS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Flomoxef Sodium and Flomoxef Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the ^1H spectrum of a solution of Flomoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate as an internal reference compound: it exhibits a single signal, A, at around δ 3.5 ppm, a single signal or a sharp multiple signal, B, at around δ 3.7 ppm, and a single signal, C, at around δ 5.2 ppm. The ratio of the integrated intensity of these signals, A : B : C, is about 3 : 2 : 1.

(5) Flomoxef Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -8 ~ -13° (1 g calculated on the anhydrous basis, a mixture of water and ethanol (99.5) (4 : 1), 50 mL, 100 mm).

pH Dissolve 0.5 g (potency) of Flomoxef Sodium in 5 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Flomoxef Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Flomoxef Sodium in a quartz crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—To 1.0 g of Flomoxef Sodium add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat carefully until the solution is colorless to pale yellow with occasional addition of 2 mL of nitric acid. After cooling, add 10 mL of ammonium oxalate TS, heat until white fumes evolve, and concentrate to 2 to 3 mL. After cooling, add water to make 10 mL, and perform the test using this solution as the test solution: the color is not more intense than that of the control solution.

Control solution—Proceed to prepare a solution in the same manner as the test solution without Flomoxef Sodium, transfer 10 mL of the solution so obtained to a generator bottle, add exactly 2 mL of standard arsenic solution, and proceed in the same manner as the test solution (not more than 2 ppm).

(4) ***1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol***—Use the test solution obtained in the Assay as the test solution. Separately, weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard. The amount of 1-(2-hydroxyethyl)-

1*H*-tetrazol-5-thiol is not more than 1.0 % of Flomoxef Sodium, calculated on the anhydrous basis.

$$\text{Amount (mg) of 1-(2-hydroxyethyl)-1}H\text{-tetrazol-5-thiol } (C_3H_6N_4OS) = \text{Amount [mg (potency)] of 1-(2-hydroxyethyl)-1}H\text{-tetrazol-5-thiol} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of *m*-cresol (3 in 1000)

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

Proceed as directed in the system suitability in the Assay.

Water Not more than 1.5 % (0.5 g, volumetric titration, back titration).

Sterility Test It meets the requirement, when Flomoxef Sodium is used in a sterile preparation. Use a diluent solution containing 0.1 % polysorbate 80 as the rinsing fluid.

Bacterial Endotoxins Less than 0.025 EU/mg (potency) of flomoxef, when Flomoxef Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Flomoxef Sodium and Flomoxef Triethylammonium RS, dissolve each in 50 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of flomoxef to that of the internal standard.

$$\text{Amount [μg (potency)] of flomoxef } (C_{15}H_{18}F_2N_6O_7S_2) = \text{Amount [μg (potency)] of Flomoxef Triethylammonium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *m*-cresol (3 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 246 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate, and 1.60 g of tetra-*n*-butylammonium bromide in water to make 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of flomoxef is about 9 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, flomoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 3 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of flomoxef to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 5 °C.

Flomoxef Sodium for Injection

Flomoxef Sodium for Injection is a preparation for injection, which is dissolved before use.
Flomoxef Sodium for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Method of Preparation Prepare as directed under Injections, with Flomoxef Sodium.

Description Flomoxef Sodium for Injection appears as white to pale yellowish white, friable masses or powder.

Identification Proceed with Flomoxef Sodium for Injection as directed in the Identification (3) under Flomoxef Sodium.

pH Dissolve an amount of Flomoxef Sodium for Injection, equivalent to 0.5 g (potency) of flomoxef sodium, in 5 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve an amount of Flomoxef Sodium for Injection, equivalent to 1.0 g (potency) of flomoxef sodium according to the labeled amount, in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol***—Use the test solution obtained in the Assay as the test solution. Separately, weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the standard solution and water to make 50 mL, and use this solution as the standard

solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard. The amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol is not more than 10 mg per g (potency) of Flomoxef Sodium for Injection.

$$\text{Amount (mg) of 1-(2-hydroxyethyl)-1}H\text{-tetrazol-5-thiol } (C_3H_6N_4OS) = \text{Amount [mg (potency)] of 1-(2-hydroxyethyl)-1}H\text{-tetrazol-5-thiol} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of *m*-cresol (3 in 1000)

Operating conditions

Proceed as directed in the operating conditions in the Assay under Flomoxef Sodium.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol obtained from 5 μL of this solution is equivalent to 3.5 to 6.5 % of that from 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 3 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard is not more than 1.0 %.

Water Not more than 1.5 % (0.5 g, volumetric titration, back titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.025 EU/mg (potency) of flomoxef.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

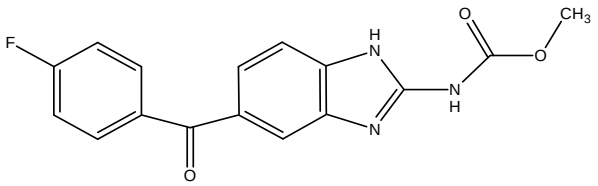
Assay Weigh accurately the mass of the contents of not less than 10 containers of Flomoxef Sodium for Injection, and calculate the average mass of the contents. Spread out thinly about 1 g of the contents in a petri dish, and allow the dish to stand in a desiccator containing a saturated solution of magnesium bromide without light exposure to equilibrate the sample to constant water content. Determine the water content with about 0.1 g of the sample according to the method described in Water. Weigh accurately an amount of the sample, equivalent to about 50 mg (potency) of flomoxef sodium according to the labeled amount, dissolve in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Flomoxef Triethylammonium RS, dissolve in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Flomoxef Sodium.

$$\text{Amount [μg (potency)] of flomoxef } (C_{15}H_{18}F_2N_6O_7S_2) = \text{Amount [μg (potency)] of Flomoxef Triethylammonium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *m*-cresol (3 in 1000)

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Flubendazole



$C_{16}H_{12}FN_3O_3$: 313.28

Methyl *N*-[6-(4-fluorobenzoyl)-1*H*-benzimidazol-2-yl]carbamate [*31430-15-6*]

Flubendazole contains not less than 99.0 % and not more than 101.0 % of flubendazole ($C_{16}H_{12}FN_3O_3$), calculated on the dried basis.

Description Flubendazole is white powder.
Flubendazole is practically insoluble in water, in dichloromethane or in ethanol (95).
Flubendazole shows polymorphism.

Identification Determine the infrared spectra of Flubendazole and Flubendazole RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity Related substances—Dissolve 0.10 g of Flubendazole in N,N-dimethylformamide to make exactly 100 mL and use this solution as the test solution. Pipet 1.0 mL of the test solutionn, add N,N-dimethylformamide to make exactly 100 mL. Pipet 5.0 mL of the this solutionn, add N,N-dimethylformamide to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions: peak area of any related substance with relative retention time between 1.2 and 1.3 is not more than that of the principal peak from the standard solution (0.25 %), and sum of areas of peaks from the test solution except the principal peak is not more than 6 times the area of the principal peak from the standard solution (1.5 %). Disregard peaks from the test solution with areas of 0.2 times of the area of the principal peak from the standard solution.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 250 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 10 cm in length, packed with base-deactivated octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase : Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 7.5 g of ammonium acetate in water to make 1000 mL.

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-15	90→75	10→25
15-30	75→45	25→55
30-32	45→10	55→90
32-37	10	90
37-38	10→90	90→10
38-42	90	10

Flow rate: 1.2 mL/minute

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

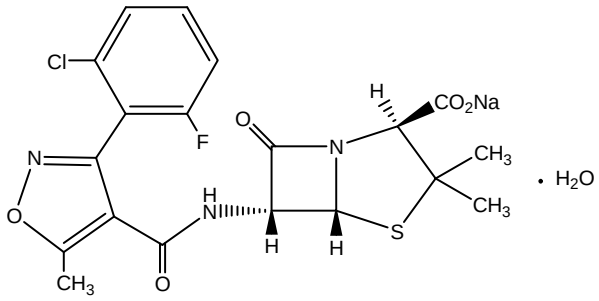
Assay Weigh accurately about 0.25 g of Flubendazole, dissolve in 3 mL of formic acid, add 50 mL of a mixture of 2-butanone and acetic acid (100) (7 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.330 mg of $C_{16}H_{12}FN_3O_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Flucloxacillin Sodium



$C_{19}H_{16}ClFN_3NaO_5S \cdot H_2O$: 493.87

Sodium(2*S*,5*R*,6*R*)-6-[[3-(2-chloro-6-fluorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*1847-24-1*]

Flucloxacillin Sodium contains not less than 827 μg (potency) per mg of flucloxacillin ($C_{19}H_{16}ClFN_3O_5S$: 453.87), calculated on the anhydrous basis.

Description Flucloxacillin Sodium appears as white to pale yellow-white crystals or crystalline powder, is odorless, and has a bitter taste.
Flucloxacillin Sodium is freely soluble in water or in methanol, soluble in ethanol (95), slightly soluble in acetone, and practically insoluble in ether, in chloroform, or in benzene.

Identification (1) To 2 mg (potency) of Flucloxacillin Sodium add 2 mg of chromotropic acid and 2 mL of sulfuric acid, and heat at 150 °C: a purple color develops 3 minutes later through green-yellow.

(2) Dissolve 0.1 g (potency) of Flucloxacillin Sodium in water to make 500 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 274 and 276 nm.

(3) Transfer 10 mg (potency) of Flucloxacillin Sodium to a hard glass tube, add a small piece of sodium metal, heat carefully and slowly, and ignite for 1 minute to disintegrate complete. After cooling, add water, shake, and centrifuge. To 0.5 mL of the clear supernatant liquid add 2 to 3 drops of a solution of zirconium-alizarin: the color of the solution changes from red to yellow.

(4) Determine the infrared spectra of Flucloxacillin Sodium and Flucloxacillin Sodium RS as directed in

the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) Dissolve separately a suitable amount each of Flucloxacillin Sodium and Flucloxacillin Sodium RS in methanol so that each mL contains 5 mg (potency), and use these solutions as the test solution and standard solution, respectively. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (5 : 1), allow the plate to stand in a well-closed container saturated with ammonia vapor for 10 minutes, then heat at 70 °C for 10 minutes. After cooling, spray evenly the coloring agent on the plate: the test solution shows an orange or red spot at the same R_f value as the standard solution. Prepare the coloring agent by dissolving 50 mg of 4-dimethyl-aminocinnamaldehyde in 10 mL of ethanol (95) and 1 mL of acetic acid (100). Prepare this solution before use.

(6) Flucloxacillin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +152 ~ +162° (0.5 g, water, 50 mL, 100 mm).

pH Dissolve 1.0 g (potency) of Flucloxacillin Sodium in 10 mL of water: the pH of this solution is between 5.0 and 7.5.

Purity (1) ***Related substances***—Weigh accurately 50 mg of Flucloxacillin Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately 50 mg of Flucloxacillin Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5.0 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Pipet 5.0 mL of the standard solution (1), add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (2). Separately, weigh accurately 5 mg of Flucloxacillin Sodium RS and 5 mg of Cloxacillin Sodium RS, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution (3). Perform the test with 20 μL each of the test solution and standard solutions (2) and (3) as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of any related substance other than the principal peak obtained form the test solution is not larger than the area of the principal peak from the standard solution (2) (not more than 1 %), and the total amount of related substances is not larger than 5 times the area of the principal peak from the standard solution (2) (not more than 5 %). Exclude any peak with an area less than 0.05 times the area of the principal peak from the standard solution (2) (0.05 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of acetonitrile and 0.02 mol/L potassium dihydrogen phosphate (25 : 75) to 5.0 with a solution of dilute sodium hydroxide.

Flow rate: About 1 mL/minute

System suitability

System performance: When the procedure is run with the standard solution (3) under the above operating conditions, cloxacillin and flucloxacillin are eluted in this order with the resolution between these peaks being not less than 2.5.

Time span of measurement: About 6 times as long as the retention time of flucloxacillin.

(2) ***Dimethylaniline***—Weigh accurately about 1.0 g of Flucloxacillin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

$$\text{Content (ppm) dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethyani line taken} \times \text{Content (\%) of dimethylan iline}}{\text{Amount (mg) of Flucloxaci llin Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography, coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen
Flow rate: 30 mL/minute

(3) ***2-Ethylhexanoate***—Dissolve 0.3 g of Flucloxacllin Sodium in 4.0 mL of 33 % hydrochloric acid solution, add 1.0 mL of the internal standard solution, shake vigorously for 1 minute, allow the layers to separate, and use the supernatant liquid as the test solution. Dissolve 75.0 mg of 2-ethylhexanoate in the internal standard solution to make 50 mL, pipet 1.0 mL of this solution, add 4.0 mL of 33 % hydrochloric acid solution, shake vigorously for 1 minute, allow the layers to separate, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 2-ethylhexanoate to that of the internal standard in each solution: the amount of 2-ethylhexanoate is not more than 0.8 %.

$$\text{Amount (\%) of 2-ethylhexanoate} = \frac{Q_T}{Q_S} \times \frac{W_S}{W_T} \times 2$$

W_S: Amount (g) of Flucloxacillin Sodium taken
W_T: Amount (g) of 2-ethylhexanoate in the standard solution

Internal standard solution—Weigh accurately 0.1 g of 3-cyclohexylpropionate, and dissolve in cyclohexane to make 100 mL.

Operating conditions
Detector: A glass column about 0.53 mm in internal diameter and about 10 m in length, coated with polyethylene glycol 20000-2-nitroterephthalate for gas chromatography 1 μm in thickness.
Column temperature: Maintain at 40 °C for 2 minutes, raise the temperature to 200 °C at 7.3 minutes at the rate of 30 °C per minute, and maintain at 200 °C until 10.3 minutes.
Injection port temperature: 200 °C
Detector temperature: 300 °C
Carrier gas: Helium
Flow rate: 10 mL/minute
System suitability
System performance: When the procedure is run with 1 μL each of the test solution and standard solution under the above operating conditions, 2-ethylhexanoate and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

Water Not more than 5.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Flucloxacillin Sodium is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Flucloxacillin Sodium is used in a sterile preparation. Weigh an appropriate amount of Flucloxacillin Sodium, make a solution so that each mL contains 6 mg (potency), and use this solution as the test solution. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

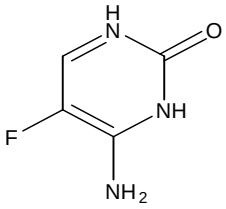
Assay Weigh accurately about 50.0 mg (potency) each of Flucloxacillin Sodium and Flucloxacillin Sodium RS, and dissolve each in the mobile phase to make exactly 50 mL. To 5 mL each of these solutions add the mobile phase to make exactly 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of flucloxacillin sodium in each solution.

$$\text{Amount [μg (potency)] of flucloxacillin } (C_{19}H_{16}ClFN_3O_5S) = \text{Amount [μg (potency)] of Flucloxacillin Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 225 nm)
Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Mobile phase: A mixture of acetonitrile, 2.7 g/L potassium dihydrogen phosphate solution (adjusted to pH 5.0 with dilute sodium hydroxide solution) (1 : 3)
Flow rate: 1.0 mL/minute
System suitability
System performance: Dissolve 5 mg of Flucloxacillin Sodium RS and 5 mg of Cloxacillin Sodium RS in the mobile phase to make 50.0 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, cloxacillin and flucloxacillin are eluted in this order with the resolution between these peaks being not less than 2.5.
System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flucloxacillin sodium is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Flucytosine



$C_4H_4FN_3O$: 129.09

6-Amino-5-fluoropyrimidin-2(1*H*)-one [*2022-85-7*]

Flucytosine, when dried, contains not less than 98.5 % and not more than 101.0 % of flucytosine ($C_4H_4FN_3O$) and not less than 14.0 % and not more than 15.5 % of Fluorine (F: 19.00).

Description Flucytosine is a white, crystalline powder and is odorless.
Flucytosine is sparingly soluble in water, slightly soluble in methanol, in acetic acid (100), in acetic anhydride or in ethanol (95) and practically insoluble in ether.
Flucytosine dissolves in 0.1 mol/L hydrochloric acid TS.
The pH of a solution of Flucytosine (1 in 100) is between 5.5 and 7.5.
Flucytosine is slightly hygroscopic.
Melting point— About 295 ºC (with decomposition).

Identification (1) Take 5 mL of a solution of Flucytosine (1 in 500), add 0.2 mL of bromine TS: a yellow-brown color of bromine TS is immediately discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is produced.

(2) Proceed with 0.1 g of Flucytosine as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. The solution responds to the Qualitative Tests (2) for fluoride.

(3) Determine the absorption spectra of the solutions of Flucytosine and Flucytosine RS in 0.1 mol/L hydrochloric acid TS (1 in 125000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Flucytosine in 100 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 1.0 g of Flucytosine in 80 mL of water by heating on a water-bath. After cooling, to 40 mL of this solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(3) ***Fluoride***—Dissolve 0.10 g of Flucytosine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1) and add water to make 20 mL. Allow the mixture to stand for 1 hour and use this solution as the test solution. Separately, transfer 4.0 mL of standard fluorine solution to a volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1). Proceed in the same manner as directed in the preparation of the test solution and use this solution as the standard solution. Transfer 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) to a volumetric flask, proceed in the same manner as directed in the preparation of the standard solution and use this solution as the blank solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 600 nm, respectively, using the blank solution as the control as directed under Ultraviolet-visible Spectrophotometry: A_T is not larger than A_S (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Flucytosine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Flucytosine according to Method 2 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 50.0 mg of Flucytosine in 5 mL of a diluted methanol (1 to 2) and use this solution as the test solution. Pipet 1 mL of this solution, add a diluted methanol (1 to 2) to make exactly 25 mL. Pipet 1 mL of this solution, add a diluted methanol (1 in 2) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for Thin-layer chromatography. Develop the chromatogram with a mixture of ethyl acetate, methanol and water (5 : 3 : 2) to a distance of about 12 cm, air-dry the plate and observe the spots under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay (1) ***Flucytosine***—Weigh accurately about 0.2 g of Flucytosine, previously dried, dissolve in 40 mL of acetic acid (100), add 100 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in

Titrimetry). Perform a blank determination and make any necessary correction.

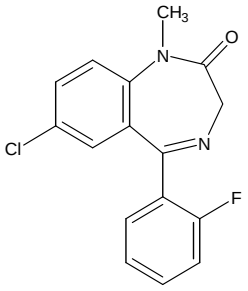
Each mL of 0.1 mol/L perchloric acid VS
= 12.909 mg of $C_4H_4FN_3O$

(2) ***Fluorine***—Weigh accurately about 10 mg of Flucytosine, previously dried and proceed as directed in the determination of fluorine under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fludiazepam



$C_{16}H_{12}ClFN_2O$: 302.73

7-Chloro-5-(2-fluorophenyl)-1-methyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one [*3900-31-0*]

Fludiazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of fludiazepam ($C_{16}H_{12}ClF\ N_2O$).

Description Fludiazepam appears as white to pale yellow crystals or crystalline powder.
Fludiazepam is very soluble in chloroform, freely soluble in methanol, in ethanol, in acetic acid (100) or in ether and practically insoluble in water.

Identification (1) Prepare the test solution with 10 mg of Fludiazepam as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests (2) for fluoride.

(2) Determine the absorption spectra of the solutions of Fludiazepam and Fludiazepam RS in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. And determine spectra of the solutions of Flueliazepam and Fludiazepam RS in methonol (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths

(3) Determine the infrared spectrum of Fludiazepam and Fludiazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Fludiazepam as directed under the Flame Coloration Test (2): a green color is observed.

Melting Point 91 ~ 94 ºC.

Purity (1) ***Chloride***—Dissolve 1.0 g of Fludiazepam in 50 mL of ether, add 50 mL of water and shake. Separate the water layer, wash with two 20 mL volumes of ether and filter the water layer. To 20 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Fludiazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Fludiazepam in 20 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 50 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (10 : 7) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, in vacuum, 60 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

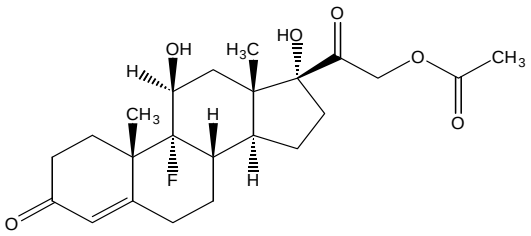
Assay Weigh accurately about 0.5 g of Fludiazepam, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.273 mg of $C_{16}H_{12}ClFN_2O$

Containers and Storage ***Containers***—Tight con-

tainers.

Fludrocortisone Acetate



$C_{23}H_{31}FO_6$: 422.49

2-((8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-9-fluoro-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)-2-oxoethyl acetate [*514-36-3*]

Fludrocortisone Acetate, when dried, contains not less than 97.0 % and not more than103.0 % of fludrocortisone acetate ($C_{23}H_{31}FO_6$).

Description Fludrocortisone Acetate appears as white to pale yellow crystals or crystalline powder, is odorless or has slight odor.
Fludrocortisone Acetate is hygroscopic.
Fludrocortisone Acetate is sparingly soluble in ethanol (95) or in chloroform, slightly soluble in ether, and practically insoluble in water.

Identification Determine the infrared spectra of Fludrocortisone Acetate and Fludrocortisone Acetate RS, as directed in the paste method under Infrared Spectrophotometry: both spectra similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +131 ~ +138° (after drying, 0.1 g , acetone, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Fludrocortisone Acetate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Weigh accurately about 20 mg of Fludrocortisone Acetate, dissolve in exactly 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobiole phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of any peak other than fludrocortisones acetate from the test solution is not larger than 1/4 times the peak area of fludrocortisones acetate from the standard solution, and the total area of the peaks other than fludrocortisones acetate from the test solution is not larger than 1/2 times the peak area of fludrocortisones acetate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of water and tetrahydrofuran (13 : 7)

Flow rate: Adjust the flow rate so that the retention time of fludrocortisones acetate is about 10 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of fludrocortisones acetate obtained from 20 μL of this solution is equivalent to 4.0 to 6.0 % of that from the standard solution.

System performance: Dissolve 2 mg each of Fludrocortisone Acetate and Hydrocortisone Acetate in the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, hydrocortisone acetate and fludrocortisones acetate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fludrocortisones acetate is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of fludrocortisones, beginning after the solvent peak.

Loss on Drying Not more than 1.0 % (1 g, 100 °C, in vacuum, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg each of Fludrocortisone Acetate and Fludrocortisone Acetate RS, previously dried at 100 ºC for 2 hours in vacuum and dissolve in chloroform to make 250 mL, respectively. Pipet 10.0 mL each of these solutions and dilute with chloroform to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Pipet 10 mL each of the test solution and the standard solution into a volumetric flask and add 1.0 mL of methanol solution of bluetetrazolium (dissolve 50 mg of bluetetrazolium in 10 mL of methanol) and mix. Add 1.0 mL of a mixture of tetramethylammonium hydroxide solution and methanol (1 : 4) and stand for 10 minutes. Add a mixture of hydrochloric acid and

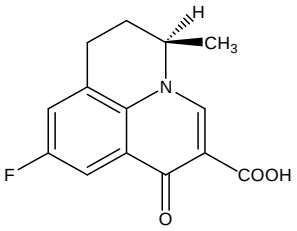
methanol (1 in 100) to make exact 25 mL. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry. Determine absorbances, A_T and A_S, of Fludrocortisone Acetate of the test solution and standard solution, respectively using as the blank prepared in the same manner with 10 mL of cholroform, at a maximum wavelength near 525 nm.

Amount (mg) of fludrocortisone acetate ($C_{23}H_{31}FO_6$)
= Amount (mg) of Fludrocortisone Acetate RS $\times \frac{A_T}{A_S}$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Flumequine



and enantiomer

$C_{14}H_{12}FNO_3$: 261.25

(*RS*)-9-Fluoro-5-methyl-1-oxo-1,5,6,7-tetrahydropyrido[3,2,1-ij]quinoline-2-carboxylic acid [*42835-25-6*]

Flumequine containes not less than 99.0 % and not more than 101.0 % of flumequine ($C_{14}H_{12}FNO_3$), calculated on the dried basis.

Description Flumequine is a white, fine crystalline powder.
Flumequine is sparingly soluble in dichloromethane, very slightly soluble in methanol, and practically insoluble in water.
Flumequine dissolves in dilute sodium hydroxide TS.

Identification (1) Mix 5 mg of Flumequine with 45 mg of magnesium oxide and ignite for about 5 minutes until an almost white residue is obtained. After cooling, add 1 mL of water and 2 drops of phenolphthalein TS, add about 2 mL of dilute hydrochloric acid to render the solution colorless. Filter and use the filtrate as the test solution. Add to the test solution a freshly prepared mixture of 0.1 mL of alizarin S TS and 0.1 mL of the zirconyl nitrate solution, mix, allow to stand 5 minutes and compare the color of the solution with that of the blank solution prepared in the same manner: the color of the test solution changes from red to yellow, while the color of the blank solution remains red.

Zirconyl nitrate solution—Dissolve 0.1 g of zirconyl nitrate in a mixture of 60 mL of hydrochloric acid and 40 mL of water.

(2) Determine the infrared spectra of Flumequine and Flumequine RS as directed in the potassiumn bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 5 mg of Flumequine in dichloromethane to make 10 mL and use this solution as the test solution. Separately, dissolve 5 mg of Flumequine RS in dichloromethane to make 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), water and 9 mol/L ammonia solution TS (90 : 10 : 10) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the test solution and the standard solution has the same R_f value.

9 mol/L ammonia solution—To 67 g of 13.5 mol/L ammonia solution, add water to make 100 mL.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10° (5.0 g, sodium hydroxide TS, 50 mL, 100mm).

Purity (1) ***Clarity and color of solution***—Dissolve 5.0 g of Flumequine in 0.5 mol/L sodium hydroxide TS to make 50 mL: the solution is clear.

(2) ***Heavy metals***—Proceed with 2.0 g of Flumequine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) **Related substances**—Dissolve 35.0 mg of Flumequine in N,N-dimethylformamide to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 5.0 mg each of flumequine RS and flumequine related substance I RS [(*RS*)-9-fluoro-5-methyl-1-oxo-6,7-dihydro-1*H*,5*H*-benzo[i.j]quinolizine-2-carboxylate (flumequine ethyl ester)] in *N,N*-dimethylformamide to make exactly 100 mL and use this solution as the standard solution (1). To 1.0 mL of the test solution, add *N,N*-dimethylformamide to make exactly 200 mL and use this solution as the standard solution (2). Perform the test with 10 μL each of the blank solution (dimethylformamide), the test solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions: area of any peak other than the principal peak from the test solution is not more than that of the principal peak from the standard solution (2) (0.5 %), and sum of areas of peaks other than the principal peak from the test

solution is not more than 2 times the area of the principal peak from the standard solution (2) (1.0 %). Disregard peaks from *N,N*-dimetylformamide and the test solution with areas of 0.1 times of the area of the principal peak from the standard solution (2).

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 250 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase : A mixture of the potassium dihydrogen phosphate buffer and methanol (51 : 49)

Flow rate : 0.8 mL/minute

System suitability

System performance: When the procedure is run with the standard solution (1) according to the above operating conditions, retention times of flumequine related substance I RS and flumequine are about 11 minutes and 13 minutes, respectively and the resolution between these peaks is not less than 2.0.

Potassium dihydrogen phosphate buffer—Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

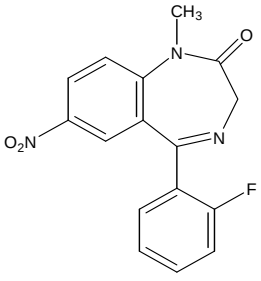
Residue on Ignition Not more than 0.1 % (0.5 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Flumequine, dissolve in 50 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS = 26.126 mg of $C_{14}H_{12}FNO_3$

Containers and Storage ***Containers***—Well-closed containers.

Flunitrazepam



$C_{16}H_{12}FN_3O_3$: 313.28

5-(2-Fluorophenyl)-1-methyl-7-nitro-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one [*1622-62-4*]

Flunitrazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of flunitrazepam ($C_{16}H_{12}FN_3O_3$).

Description Flunitrazepam is a white to pale yellow crystalline powder.

Flunitrazepam is freely soluble in acetic acid (100), soluble in acetic anhydride or in acetone, slightly soluble in ethanol (99.5) or in ether and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Flunitrazepam and Flunitrazepam RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Flunitrazepam and Flunitrazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 168 ~ 172 ºC.

Purity (1) ***Chloride***—Take 1.0 g of Flunitrazepam, add 50 mL of water, allow to stand for 1 hour with occasional stirring and filter. To 20 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test with this solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Flunitrazepam according to Method 4 using a platinum crucible and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50.0 mg of Flunitrazepam in 10 mL of acetone and use this solution as the test solution. Pipet 2 mL of the test solution and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, ether and ammonia solution (28) (200 : 100 : 3) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): numbers of the spots other than the principal spot from the test solution are not more

than 2 and they are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

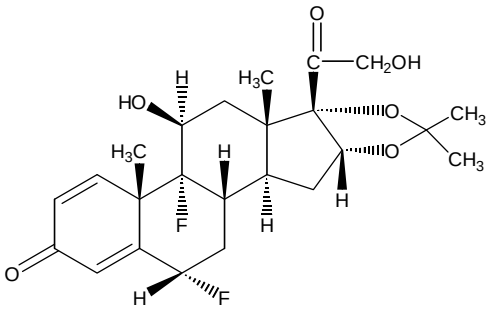
Assay Weigh accurately about 0.5 g of Flunitrazepam, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.328 mg of $C_{l6}H_{12}FN_3O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fluocinolone Acetonide



$C_{24}H_{30}F_2O_6$: 452.49

2-{(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*S*)-6,9-difluoro-11-ihydroxy-16,17-[(2-propylidene)bis(oxy)]-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl)}-2-oxoethanol [*67-73-2*]

Fluocinolone Acetonide, when dried, contains not less than 97.0 % and not more than 102.0 % of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$).

Description Fluocinolone Acetonide appears as white crystals or crystalline powder and is odorless.
Fluocinolone Acetonide is freely soluble in acetic acid (100) or acetone, soluble in ethanol (95) or ethanol (99.5), sparingly soluble in methanol or chloroform, slightly soluble in acetonitrile, very slightly soluble in ether, and practically insoluble in water.

Melting point—266 ~ 274 ºC (with decomposition).

Identification (1) Take 2 mg of Fluocinolone Acetonide, add 2 mL of sulfuric acid: a yellow color is observed.

(2) Dissolve 10 mg of Fluocinolone Acetonide in 1 mL of methanol, add 1 mL of Fehling TS and heat: a red precipitate is produced.

(3) Proceed with 10 mg of Fluocinolone Acetonide as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid. The test solution responds to the Qualitative Tests for fluoride.

(4) Determine the infrared spectra of Fluocinolone Acetonide and Fluocinolone Acetonide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit the similar intensity of absorption at the same wavenumbers. If any difference appears between spectra, dissolve the test and the RS in acetone, respectively, evaporate acetone and repeat the test on the residue in the same manner.

Specific Optical Rotation $[\alpha]_D^{20}$: +98 ~ +108° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity ***Related substances***—Dissolve about 15 mg of Fluocinolone Acetonide in 25 mL of mobile phase and use this solution as the test solution. Pipet 2 mL of this solution, add mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: the total area of all peaks other than the peak of fluocinolone acetonide from the test solution is not larger than the peak area of fluocinolone acetonide from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 ºC.

Mobile phase: A mixture of chloroform saturated with water, methanol and acetic acid (100) (200 : 3 : 2).
Flow rate: Adjust the flow rate so that the retention time of fluocinolone acetonide is about 12 minutes.

System suitability

Test for required detection: Take exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of fluocinolone acetonide obtained from 20 μL of this solution is equivalent to 4 to 6 % of that of fluocinolone acetonide obtained from 20 μL of the standard solution.

System performance: Dissolve each 15 mg of

Fluocinolone Acetonide and Triamcinolone Acetonide in 25 mL of the mobile phase. Take 5 mL of this solution, add mobile phase to make 20 mL. When the procedure is run with 20 µL of this solution, as directed under the above operating conditions, triamcinolone acetonide and fluocinolone acetonide are eluted in this order with a resolution between their peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution, as directed under the above operating conditions, the relative deviation of the peak area of fluocinolone acetonide is not more than 1.0 %.

Time span of measurement: About 2 times as long as the retention time of Fluocinolone Acetonide.

Loss on Drying Not more than 1.0 % (0.2 g, in vacuum, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.2 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Fluocinolone Acetonide and Fluocinolone Acetonide RS, previously dried, dissolve in 40 mL of methanol, add exactly 10 mL of the internal standard solution, add water to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluocinolone acetonide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fluocinolone acetonide } (C_{24}H_{30}F_2O_6) = \text{Amount (mg) of Fluocinolone Acetonide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 2500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of water and acetonitrile (7 : 3).

Flow rate: Adjust the flow rate so that the retention time of Fluocinolone Acetonide is about 20 minutes.

System suitability

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile and add water to make 100 mL. When the precedure is run with 20 µL of this solution, as directed under the above operating conditions, isopropyl *p*-hydroxybenzoate and propyl *p*-hydroxy-benzoate are eluted in this order with a resolution between their peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 µL each of the standard solution, as directed under the above operating conditions, the relative deviation of the peak area of fluocinolone acetonide is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fluocinolone Acetonide Cream

Fluocinolone Acetonide Cream contains not less than 90.0 % and not more than 110.0 % of the labeled amount of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$: 452.49).

Method of Preparation Prepare as directed under Creams, with Fluocinolone Acetonide.

Identification Weigh accurately a portion of Fluocinolone Acetonide Cream, equivalent to about 0.5 mg of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$) according to the labeled amount, transfer to a centrifugal tube, suspend with 5 mL of water, add 10 mL of chloroform, shake and centrifuge. Discard the water layer, add 10 mL of water, shake and centrifuge. Dehydrate 2 mL of chloroform extracts with about 0.2 g of anhydrous sodium sulfate and use this solution as the test solution. Separately, dissolve 5 mg of Fluocinolone Acetonide RS in 100 mL of chloroform and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 50 µL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (2 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the test solution shows the same R_f value and color as the principal spot from the standard solution.

Assay Weigh accurately a portion of Fluocinolone Acetonide Cream, equivalent to about 0.75 mg of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$) according to the labeled amount, add about 10 mL of acetonitrile and warm in the water-bath until dissolved. Transfer to a 25 mL volumetric flask with aid of three 2 mL volumes of acetonitrile. To this solution, add 3.0 mL of the internal standard solution and 5.0 mL of water, shake, add acetonitrile to make 25 mL, cool in the ice-bath, centrifuge and use the clear supernatant liquid as the test solution. Separately, weigh accurately a portion of Fluocinolone

Acetonide RS, previously dried at 105 ºC for 3 hours, dissolve in acetonitrile to obtain a solution having a known concentration of about 300 μg per mL. Pipet 5 mL of this solution, add 6.0 mL of the internal standard solution and 15.0 mL of water, add acetonitrile to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluocinolone acetonide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fluocinolone acetonide } (C_{24}H_{30}F_2O_6) = \text{Amount (mg) of Fluocinolone Acetonide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of norethindrone in acetonitrile (2 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (5 : 3).

Flow rate: 2 mL/minute.

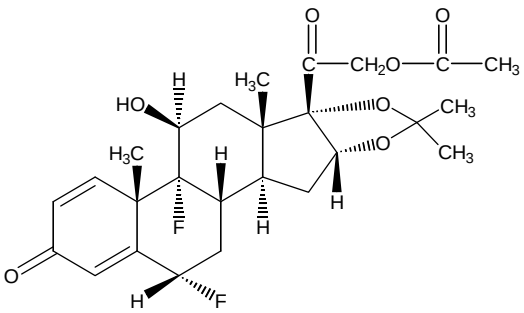
System suitability

System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, the resolution between peaks of the internal standard and fluocinolone acetonide is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, as diredcted under the above operating conditions, the relative deviation of the ratios of the peak area of fluocinolone acetonide to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Fluocinonide



$C_{26}H_{32}F_2O_7$: 494.53

2-{(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*S*)-6,9-difluoro-11-hydroxy-16,17-[(2-propylidene)bis(oxy)]-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl)}-2-oxoethyl acetate [*356-12-7*]

Fluocinonide, when dried, contains not less than 97.0 % and not more than 103.0 % of fluocinonide ($C_{26}H_{32}F_2O_7$).

Description Fluocinonide appears as white crystals or crystalline powder.

Fluocinonide is sparingly soluble in chloroform, slightly soluble in acetonitrile, in methanol, in ethanol (95) or in ethyl acetate, very slightly soluble in ether, and practically insoluble in water.

Identification (1) Take 10 mg of Fluocinonide, add 4 mL of water and 1 mL of Fehling's TS and heat: a red precipitate is produced.

(2) Prepare the test solution with 10 mg of Fluocinonide as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests for fluoride.

(3) Determine the absorption spectra of solutions of Fluocinonide and Fluocinonide RS, respectively, in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Fluocinonide and Fluocinonide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve the test and the RS in ethyl acetate, respectively, evaporate the ethyl acetate and repeat the test on the residue in the same manner.

Specific Optical Rotation $[\alpha]_D^{20}$: +81 ~ +89° (after drying, 0.2 g, chloroform, 20 mL, 100 mm).

Purity ***Related substances***—Dissolve 10 mg of Fluocinonide in 2 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (97 : 3) to a distance of about 12 cm and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g, platinum crucible).

Assay Weigh accurately about 20 mg of Fluocinonide and fluocinonide RS, previously dried, dissolve each in 50 mL of acetonitrile, to each add exactly 8.0 mL of the internal standard solution and water to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluocinonide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fluocinonide } (C_{26}H_{32}F_2O_7) = \text{Amount (mg) of Fluocinonide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl benzoate in acetonitrile (1 in 100).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of acetonitrile and water (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of fluocinonide is about 8 minutes.

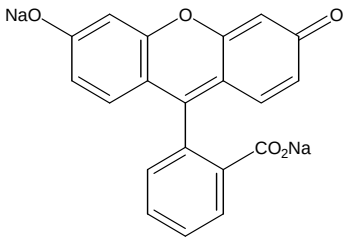
System suitability

System performance: When the procedure is run with 20 μL of the standard solution, as directed under the above operating conditions, fluocinonide and the internal standard are eluted in this order with a resolution between their peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions and the relative deviation of the peak area of fluocinonide is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Fluorescein Sodium



$C_{20}H_{10}Na_2O_5$: 376.27

2-(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid
[*518-47-8*]

Fluorescein Sodium contains not less than 98.5 % and not more than 101.0 % of fluorescein sodium ($C_{20}H_{10}Na_2O_5$), calculated on the dried basis.

Description Fluorescein Sodium is an orange powder, is odorless and tasteless.
Fluorescein Sodium is freely soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.
Fluorescein Sodium is hygroscopic.

Identification (1) Take a solution of Fluorescein Sodium (1 in 100) having a strong green fluorescence, add a large volume of water: the fluorescence remains. Acidify the solution with hydrochloric acid: the fluorescence disappears. Then render the solution alkaline with sodium hydroxide TS: the fluorescence reappears.

(2) Place 1 drop of a solution of Fluorescein Sodium (1 in 2000) on a piece of filter paper: a yellow spot develops. Expose the spot, while moist, to the vapor of bromine for 1 minute and then to ammonia vapor: the yellow color of the spot changes to red.

(3) Char 0.5 g of Fluorescein Sodium by ignition, cool, mix the residue with 20 mL of water and filter: the filtrate responds to the Qualitative Tests for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Fluorescein Sodium in 10 mL of water: the solution is clear and shows a red color.

(2) ***Chloride***—Dissolve 0.15 g of Fluorescein Sodium in 20 mL of water, add 6 mL of dilute nitric acid and water to make 30 mL and filter. Take 20 mL of the filtrate, add 2 mL of dilute nitric acid and water

to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.355 %).

(3) ***Sulfate***—Dissolve 0.20 g of Fluorescein Sodium in 30 mL of water, add 2.5 mL of dilute hydrochloric acid and water to make 40 mL and filter. To 20 mL of the filtrate, add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480 %).

(4) ***Zinc***—Dissolve 0.1 g of Fluorescein Sodium in 10 mL of water, add 2 mL of hydrochloric acid and filter. To the filtrate, add 0.1 mL of potassium hexacyanoferrate (III) TS: no turbidity is produced immediately.

(5) ***Related substances***—Dissolve 0.20 g of Fluorescein Sodium in 10 mL of methanol and use this solution as the test solution. Perform the test with this solution as directed under Thin-layer Chromatography. Spot 5 μL of the test solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30 : 15 : 1) to a distance of about 10 cm and air-dry the plate: any colored spot other than the principal spot does not appear.

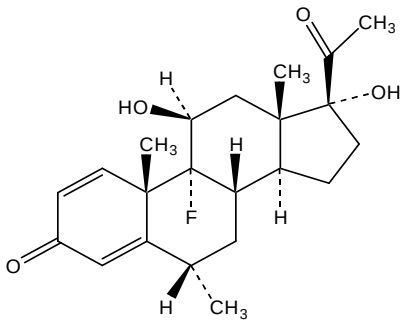
Loss on Drying Not more than 10.0 % (1 g, 105 ºC, constant mass).

Assay Transfer 0.5 g of Fluorescein Sodium, accurately weighed, to a separator. Dissolve in 20 mL of water, add 5 mL of dilute hydrochloric acid and extract the solution with four 20 mL volumes of a mixture of 2-methyl-1-propanol and chloroform (1 : 1). Wash each extract with 10 mL of water. Evaporate the combined extracts on a water-bath with the aid of a current of air. Dissolve the residue in 10 mL of ethanol (99.5), evaporate the solution on a water-bath to dryness, dry the residue at 105 ºC for 1 hour and weigh as fluorescein ($C_{20}H_{12}O_5$: 332.31).

Amount (mg) of $C_{20}H_{10}Na_2O_5$
= Amount (mg) of fluorescein ($C_{20}H_{12}O_5$) × 1.1323

Containers and Storage ***Containers***—Tight containers.

Fluorometholone



$C_{22}H_{29}FO_4$: 376.46

(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-17-Acetyl-9-fluoro-11,17-dihydroxy-6,10,13-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*426-13-1*]

Fluorometholone, when dried, contains not less than 97.0 % and not more than 103.0 % of fluorometholone ($C_{22}H_{29}FO_4$).

Description Fluorometholone is as a white to pale yellowish white, crystalline powder and is odorless.
Fluorometholone is freely soluble in pyridine, slightly soluble in methanol, in ethanol (95) or in tetrahydrofuran, and practically insoluble in water or in ether.

Identification (1) Proceed with 7 mg of Fluorometholone as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the liquid responds to the Qualitative Tests (2) for fluoride.

(2) Determine the absorption spectra of solutions of Fluorometholone and Fluorometholone RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Fluorometholone and Fluorometholone RS, previously dried, as directed in the potassiumn bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +52 ~ +60° (after drying, 0.1 g, pyridine, l0 mL, 100mm).

Purity (1) ***Heavy metals***—Proceed with l.0 g of Fluorornetholone according to Method 3, and performn the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 20 mg of Fluorometholone in 10 mL of tetrahydrofuran, and use this solution as the test solution. Pipet 1 mL of the test

solution, add tetrahydrofuran to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 25 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone and methanol (45 : 5 : 1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.2 g, in vacuum, P_2O_5, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (0.2 g, platinum crucible).

Assay Weigh accurately about 0.10 g each of Fluorometholone and Fluorometholone RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and diluted methanol (7 in 10) to make 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the ratios, Q_T and Q_S, of the peak area of fluorometholone to that of the internal standard.

$$\text{Amount (mg) of fluorometholone } (C_{22}H_{29}FO_4) = \text{Amount (mg) of Fluorometholone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 25 to 30 cm in length, packed with ociadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: Diluted methanol (7 in 10).

Flow rate: Adjust the flow rate so that the retention time of fluorometholone is about 8 minutes.

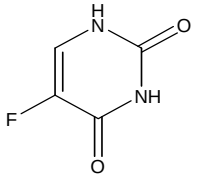
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluorometholone and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Fluorouracil



$C_4H_3FN_2O_2$: 130.08

5-Fluoropyrimidine-2,4(1*H*,3*H*)-dione [*51-21-8*]

Fluorouracil, when dried, contains not less than 98.5 % and not more than 101.0 % of fluorouracil ($C_4H_3FN_2O_2$) and not less than 13.1 % and not more than 16.1 % of fluorine (F: 19.00).

Description Fluorouracil appears as white crystals or crystalline powder and is odorless.

Fluorouracil is freely soluble in *N,N*-dimethylformamide, sparingly soluble in water, slightly soluble in ethanol (95) and practically insoluble in ether.

Melting point—About 282 ºC (with decomposition).

Identification (1) Take 5 mL of a solution of Fluorouracil (1 in 500), add 0.2 mL of bromine TS: the color of bromine TS is discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is produced.

(2) Proceed with 10 mg of Fluorouracil as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests for fluoride.

(3) Determine the absorption spectra of the solutions of Fluorouracil and Fluorouracil RS in 0.1 mol/L hydrochloric acid VS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Clarity and color of solution***—Add 20 mL of water to 0.20 g of Fluorouracil and dissolve by warming: the solution is clear and colorless.

(2) ***Fluoride***—Dissolve 0.10 g of Fluorouracil in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1) and add water to make 20 mL. Allow to stand for 1 hour and use this solution as the test solution. Separately, transfer 1.0 mL of standard fluorine solution to a volumetric flask, add

5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) and add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1 : 1 : 1). Proceed in the same manner as directed for the preparation of the test solution and use this solution as the standard solution. Perform the test as directed under Ultraviolet-visible Spectrophotometry, using a solution, prepared with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the test solution at 600 nm is not larger than that of the standard solution (not more than 0.012 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Fluorouracil according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—To 1.0 g of Fluorouracil in a crucible, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10), ignite the ethanol to burn and incinerate by strong heating at 750 to 850 ºC. If a carbonized substance remains in this method, moisten with a small amount of nitric acid and incinerate by strong heating. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water-bath, use this solution as the test solution and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Fluorouracil in 10 mL of water and use this solution as the test solution. Measure exactly 1 mL of this solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (7 : 4 : 1) to a distance of about 12 cm, air-dry the plate and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 80 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay (1) ***Fluorouracil***—Weigh accurately about 0.2 g of Fluorouracil, previously dried, dissolve in 20 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.008 mg of $C_4H_3FN_2O_2$

(2) ***Fluorine***—Weigh accurately about 4 mg of Fluorouracil, previously dried and proceed as directed in the determination of fluorine under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and Storage ***Containers***—Tight containers.

Fluorouracil Cream

Fluorouracil Cream contains not less than 90.0 % and not more than 110.0 % of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$: 130.08).

Method of Preparation Prepare as directed under Creams, with Fluorouracil. Sodium hydroxide may be added to adjust the pH.

Identification Transfer, accurately weighed, a portion of fluorouracil Cream, equivalent to about 5 mg of Fluorouracil, to a stoppered conical flask, add 50 mL of ethanol (95), shake until dissolved and use this solution as the test solution. Separately, weigh 5 mg of fluorouracil RS, dissolve in 50 mL of ethanol (95) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 100 μL each of the test solution and the standard solution (5 times with 20 μL) on a line about 3 cm from the bottom edge of a thin-layer chromatographic plate coated with a 25-m layer of silica gel, develop the chromatogram with a mixture of ethyl acetate, methanol and ammonia solution (28) (75 : 25 : 1) to a distance of about 15 cm and remove and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots from the test solution and the standard solution show the same R_f value.

Assay Weigh accurately a portion of Fluorouracil Cream, equivalent to about 10 mg of fluorouracil ($C_4H_3FN_2O_2$), add 20 mL of methanol, shake and add water to make 100 mL. Pipet 1 mL of this solution, add water to make 10 mL, filter and use this filtrate as the test solution. Separately, weigh accurately about 10 mg of Fluorouracil RS, previously dried, dissolve in water to make 100 mL, pipet 1 mL of this solution, add water to make 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the the operating conditions under Fluorouracil Injection: determine the peak areas, A_T and A_S, of Fluorouracil, for the test solution and the standard solution, respectively.

Amount (mg) of fluorouracil ($C_4H_3FN_2O_2$)

$$= \text{Amount (mg) of Fluorouracil RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Fluorouracil Injection

Fluorouracil Injection is an aqueous solution for injection. Fluorouracil Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$: 130.08).

Method of Preparation Prepare as directed under Injections, with Fluorouracil by adding sodium hydroxide.

Description Fluorouracil Injection is a colorless, clear liquid.

Identification (1) The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation, both relative to the internal standard, as obtained in the Assay.

(2) Measure a volume of Fluorouracil Injection, equivalent to about 0.1 g of Fluorouracil according to the labeled amount, acidify with acetic acid (100) carefully, shake and precipitate the Fluorouracil. Collect the precipitate, wash with 1 mL of water and dry in a desiccator (P_2O_5) at 80 ºC for 4 hours. Determine the infrared spectra of the precipitate and Fluorouracil RS, respectively, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Proceed as directed in the Identification (1) under Fluorouracil.

pH 8.6 ~ 9.4.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.33 EU/mg of fluorouracil.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injection It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay Pipet a volume of Fluorouracil Injection, equivalent to about 50 mg of fluorouracil ($C_4H_3FN_2O_2$) according to the labeled amount and add water to make 100 mL. Pipet 5 mL of this solution, add water to make 250 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Fluorouracil RS, previously dried, dissolve in water to make 100 mL, pipet 5 mL of this solution, add water to make 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions: determine the peak areas, A_T and A_S, of fluorouracil for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fluorouracil } (C_4H_3FN_2O_2) = \text{Amount (mg) of Fluorouracil RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Water.

Flow rate: 1.0 mL/minute.

System suitability

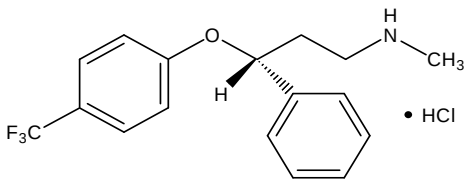
System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, the number of theoretical plates of the peak of fluorouracil is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluorouracil is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, in a dark place, and avoid freezing.

Fluoxetine Hydrochloride



$C_{17}H_{17}F_3NO \cdot HCl$: 345.79

N-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy] propan-1-aminehydrochloride [*56296-78-7*]

Fluoxetine Hydrochloride contains not less than 98.0 % and not more than 102.0 % of fluoxetine hydrochloride ($C_{17}H_{17}F_3NO \cdot HCl$), calculated on the anhydrous basis.

Description Fluoxetine Hydrochloride is a white, crystalline powder.
Fluoxetine Hydrochloride is freely soluble in ethanol (95) or in methanol, sparingly soluble in dichloromethane, and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Fluoxetine Hydrochloride and Fluoxetine Hydrochloride RS, previously dried, as directed in the potassiumn bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Fluoxetine Hydrochloride (1 in 50) uid responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with l.0 g of Fluoxetine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve about 56 mg of Fluoxetine Hydrochloride, accurately weighed, in the mobile phase to make exactly 10 mL and use this solution as the test solution (1). To exactly 2.0 mL of the test solution (1), add the mobile phase to make exactly 10 mL and use this solution as the test solution (2). Perform the test with 10 μL each of the test solution (1) and the test solution (2) as directed under Liquid Chromatography according to the following conditions. Measure the area of the peak corresponding to fluoxetine related substance I {*N*-methyl-3-pheny-[(α,α,α-(trufluoro-m-tolyl)oxy)propylamine hydrochloride], A_T and the area of peak of fluoxetine, A_U, from the test solution (2) and calculate the area of peak of each related substances, A_i, and total area of peaks of related substances other than the principal peak form the test solution (1): the amount of the fluoxetine related substance I is not more than 0.15 %, the amount of α-[2-(methylamino)ethyl] benzene-methanol is not more than 0.25 %, the amount of the fluoxetine related substance II {*N*-methyl-3-pheny-lpropylamine} is not more than 0.25 %, the amount of each other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.5 %.

$$\text{Amount (\%) of fluoxetine related substance I} = 100 \times \frac{A_T}{A_T + A_U}$$

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S + 5A_U}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with base-deactivated octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of the triethylamine buffer, stabilizer-free tetrahydrofuran, and methanol (6 : 3 : 1).

Flow rate: 1 mL/minute.

System suitability

System performance: Dissolve about 22 mg of Fluoxetine Hydrochloride RS, accurately weighed, in 10 mL of 1 mol/L sulfuric acid TS, heat at 80 °C for 3 hours, cool and transfer 0.4 mL of this solution to a 25-mL volumetric flask. Add 28 mg of Fluoxetine Hydrochloride RS, 1 mg of fluoxetine related substance I and 1 mg of fluoxetine related substance II, and add the mobile phase to make exactly 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, relative retention times of α-[2-(methylamino) ethyl]benzenemethanol, fluoxetine related substance II, fluoxetine related substance I, fluoxetine and 4-trifluoromethylphenol are about 0.24, 0.27, 0.94, 1.0 and 2.17, respectively, and the ratio of the height of the fluoxetine related substance I peak to the depth of the valley between the fluoxetine and fluoxetine related substance I peaks measured from the fluoxetine related substance I peak height is not more than 1.1.

Time span of measurement: At least 2 times as long as the retention time of fluoxetine.

Triethylamine buffer—Add 10 mL of triethylamine to 980 mL of water and adjust to a pH of 6.0 with phosphoric acid.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Assay Dissolve about 10 mg each of Fluoxetine Hydrochloride and Fluoxetine Hydrochloride RS, accurately weighed, in the mobile phase to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and measure the areas of the pricipal peaks obtained from each solutions, A_T and A_S.

$$\text{Amount (mg) of fluoxetine hydrochloride } (C_{17}H_{17}F_3NO\cdot HCl) = \text{Amount (mg) of Fluoxetine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with base-deactivated octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of the triethylamine buffer, stabilizer-free tetrahydrofuran, and methanol (6 : 3 : 1).

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run

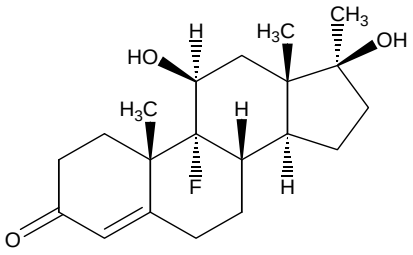
with 10 μL of the standard solution under the above operating conditions, the symmetry factor of the fluoxetine peak is not more than 2.0.

System reapetibility: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluoxetine is not more than 2.0 %.

Triethylamine buffer—Add 10 mL of triethylamine to 980 mL of water and adjust to a pH of 6.0 with phosphoric acid.

Containers and Storage ***Containers***—Tight containers.

Fluoxymesterone



$C_{20}H_{29}FO_3$: 336.44

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,17*R*)-17-Acetyl-9-fluoro-11,17-dihydroxy-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*76-43-7*]

Fluoxymesterone, when dried, contains not less than 97.0 % and not more than 102.0 % of fluoxymesterone ($C_{20}H_{29}FO_3$).

Description Fluoxymesterone appears as white crystals or crystalline powder, and is odorless.
Fluoxymesterone is sparingly soluble in methanol, slightly soluble in ethanol (95) or chloroform, very slightly soluble in ether, and practically insoluble in water.

Identification (1) Dissolve 5 mg of Fluoxymesterone in 2 mL of sulfuric acid: a yellow color is observed.

(2) Prepare the test solution with 10 mg of Fluoxymesterone as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests (2) for fluoride.

(3) Determine the absorption spectra of solutions of Fluoxymesterone and Fluoxymesterone RS, respectively, in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Fluoxymesterone and Fluoxymesterone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit the similar intensity of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve the test and the references standard in ethanol (99.5), respectively, evaporate the ethanol (99.5) and the test on the residue in the same manner.

Specific Optical Rotation $[\alpha]_D^{20}$: +104 ~ +112° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Fluoxymesterone according to Method 2 and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 30 mg of Fluoxymesterone in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, ethanol (95) and ethyl acetate (3 : 1 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.2 % (0.5 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fluoxymesterone and Fluoxymesterone RS, previously dried, dissolve each in the internal standard solution to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluoxymesterone to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fluoxymesterone } (C_{20}H_{29}FO_3) = \text{Amount (mg) of Fluoxymesterone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methylprednisolone in a mixture of chloroform and methanol (19 : 1) (1 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol and acetic acid (100) (95 : 95 : 14 : 7 : 6).

Flow rate: Adjust the flow rate so that the retention time of Fluoxymesterone is about 9 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, fluoxymesterone and the internal standard are eluted in this order with a resolution between their peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, as directed under the above operating conditions, the relative deviation of the peak area of fluoxymesterone is not more than 1.5 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Fluoxymesterone Tablets

Fluoxymesterone Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of fluoxymesterone ($C_{20}H_{29}FO_3$: 336.44).

Method of Preparation Prepare as directed under Tablets, with Fluoxymesterone.

Identification Weigh equivalent to about 20 mg of fluoxymesterone ($C_{20}H_{29}FO_3$) to a portion of powdered Fluoxymesterone Tablets, add 20 mL of hot chloroform, shake and decant the clear supernatant liquid through a filter. Repeat the extraction with two 20 mL volumes of hot chloroform, combine the extracts, evaporate on the water-bath to dryness, dissolve the residue in 5 mL of acetone, decant the clear supernatant liquid and filter the precipitate produced after adding 20 mL of water. Dissolve the precipitate in 5 mL of acetone and filter the precipitate produced after adding 20 mL of water. Perform the test with the precipitate, dried at 105 ºC for 3 hours, as directed in the Identification (4) under Fluoxymesterone.

Dissolution Test Perform the test with 1 tablet of Fluoxymesterone Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid TS as the dissolution solution. Take the dissolved solution after 60 minutes from starting of the test, filter, take 20 mL of the filtrate, add 2.0 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 28 mg of Fluoxymesterone RS, and add ethanol (95) to make exactly 25 mL. Pipet 5 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 250 mL. Pipet 5 mL of this solution and 2.0 mL of the internal standard solution, add 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluoxymesterone to that of the internal standard for the test solution and the standard solution, respectively.

The dissolution rate of Fluoxymesterone Tablets in 60 minutes is not less than 70 %.

Internal standard solution—A solution of Norethindrone in ethanol (95) (4.6 in 100000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (58 : 42).

Flow rate: 3 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution, as directed under the above operating conditions, fluoxymesterone and norethindrone are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the ratio of the peak area of fluoxymesterone to that of the internal standard is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement of the Content Uniformity Test when the test is performed according to the following method.

Transfer 1 Fluoxymesterone Tablets to a suitable container, add 2 mL of water and sonicate for about 30 minutes until the tablet completely disintegrates. Add the internal standard solution to obtain a solution having a known concentration of 250 μg per mL of fluoxymesterone ($C_{20}H_{29}FO_3$) and proceed as directed in the Assay under Fluoxymesterone.

Assay Weigh accurately and powder not less than 20

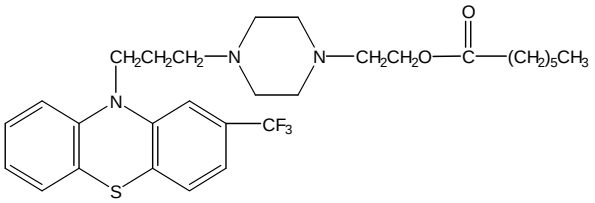
Fluoxymesterone Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of fluoxymesterone ($C_{20}H_{29}FO_3$), add 20 mL of the internal standard solution. Sonicate for 10 minutes and mix for 15 minutes. Filter this solution and use this solution as the test solution. Proceed as directed in the Assay under Fluoxymesterone.

$$\text{Amount (mg) of fluoxymesterone } (C_{20}H_{29}FO_3) = \text{Amount (mg) of Fluoxymesterone RS} \times \frac{Q_T}{Q_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Fluphenazine Enanthate



$C_{29}H_{38}F_3N_3O_2S$: 549.69

2-[4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]piperazin-1-yl]ethylheptanoate [*2746-81-8*]

Fluphenazine Enanthate, when dried, contains not less than 98.5 % and not more than 101.0 % of fluphenazine enanthate ($C_{29}H_{38}F_3N_3O_2S$).

Description Fluphenazine Enanthate is a pale yellow to yellowish orange, viscous liquid. Fluphenazine Enanthate is generally clear and can be opaque by producing crystal.

Fluphenazine Enanthate is freely soluble in methanol or in ether, soluble in ethanol (95) or in acetic acid (100), and practically insoluble in water.

Identification (1) Prepare the test solution with 10 mg of Fluphenazine Enanthate as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests for fluoride.

(2) Dissolve 2 mg each of Fluphenazine Enanthate and Fluphenazine Enanthate RS separately in 200 mL of a solution of hydrochloric acid in methanol (17 in 2000) and determine the absorption spectra as directed under Ultraviolet-visible Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Fluphenazine Enanthate and Fluphenazine Enanthate RS as directed in the liquid film method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Fluphenazine Enanthate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 0.25 g of Fluphenazine Enanthate in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, hexane and ammonia solution (28) (16 : 6 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution. Then spray evenly diluted sulfuric acid (1 in 2) on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, 60 ºC, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

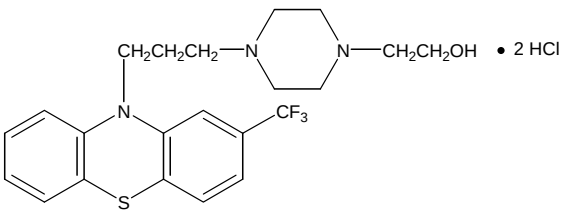
Assay Weigh accurately about 0.5 g of Fluphenazine Enanthate, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 27.485 \text{ mg of } C_{29}H_{38}F_3N_3O_2S$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fluphenazine Hydrochloride



$C_{22}H_{26}F_3N_3OS \cdot 2HCl$: 510.44

2-(4-{3-[2-(Trifluoromethyl)phenothiazin-10-yl]propy}piperazin-1-yl)ethanol dihydrochloride [*146-56-5*]

Fluphenazine Hydrochloride contains not less than 97.0 % and not more than 103.0 % of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$), calculated on the dried basis.

Description Fluphenazine Hydrochloride is a white or nearly white crystalline powder and has no odor.
Fluphenazine Hydrochloride is freely soluble in water, slightly soluble in acetone, in ethanol or in chloroform, and practically insoluble in benzene or in ether.

Melting point—225 ºC.

Identification (1) Determine the ultraviolet absorption spectra of solution of Fluphenazine Hydrochloride and Fluphenazine Hydrochloride RS in methanol (1 in 100000) as direated under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of the absorption at the same wavelengths and the respective absorbance, calculated on the dried basis, at the wavelength of a maximum absorbance at about 259 nm do not differ by more than 2.5 %.

(2) Determine the infrared spectra of Fluphenazine Hydrochloride and Fluphenazine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Fluphenazine Hydrochloride responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Weigh about 1.0 g of Fluphenazine Hydrochloride and perform a test according to the Method 2. The control solution contains 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve about 0.1 g of Fluphenazine Hydrochloride in sodium hydroxide · methanol TS to make 10 mL and use this solution as the test solution. Separately, dissolve 10 mg of Fluphenazine Hydrochloride RS in sodium hydroxide · methanol TS to make 10 mL. Pipet 0.1 mL, 0.5 mL, 1 mL and 2 mL of test solution, add sodium hydroxide · methanol TS to make exactly 10 mL and use this solution as the standard solution (1), standard solution (2), standard solution (3), standard solution (4). Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution (1), standard solution (2), standard solution (3), standard solution (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, cyclohexane and diethylamine (40 : 15 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): prepare intensities of the spots other than the principal spot from the test solution and the spot from the standard solutions, the the sum of the related substances is not less than 2.0 %.

Loss on Drying Not more than 1.0 % (1 g, 65 ºC, 3 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Dissolve about 0.12 g of Fluphenazine Hydrochloride, accurately weighed, in a mobile phase without triethylamine to make 100 mL exactly. Pipet 5 mL of this solution, dilute in mobile phase without triethylamine to make 100 mL exactly. Filter and use this solution as the test solution. Separately, dissolve about 0.12 g of Fluphenazine Hydrochloride RS, accurately weighed, in a mobile phase without triethylamine to make 100 mL exactly. Pipet 5 mL of this solution, dilute in mobile phase without triethylamine to make 100 mL exactly. Filter and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the, the peak areas of fluphenazine, A_T and A_S, for the test solution and the standard solution.

$$\text{Amount (mg) of fluphenazine hydrochloride } (C_{22}H_{26}F_3N_3OS) = \text{Amount (mg) of Fluphenazine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 12.5 cm in length, having octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Filter a mixture of 0.05 mol/L of potassium dihydrogen phosphoric acid (controlled by phosphoric acid to pH 2.5), acetonitrile and methanol (40 : 30 : 30) and add triethylamine to make 0.2 %.

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution, as directed under the above operating conditions, symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution and the relative standard deviation of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Fluphenazine Hydrochloride Tablets

Fluphenazine Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$: 510.44).

Method of Preparation Prepare as directed under Tablets, with Fluphenazine Hydrochloride.

Identification Transfer a portion of finely powdered Tablets, equivalent to about 10 mg of Fluphenazine Hydrochloride according to the labeled amount, and 10 mg of Fluphenazine Hydrochloride RS separately into two separators. Add 5 mL of water and 20 mL of diluted hydrochloric acid (1 in 120) to each separator, shake for 10 minutes and to each mixture, add 20 mL of chloroform saturated with sodium carbonate solution (1 in 10). Extracts each mixture with five 20 mL volumes of chloroform-washed cotton filters into separate beakers. Evaporate the extracts on a steam-bath to dryness and dissolve the residues separately in 0.5 mL of a mixture of methanol and water (4 : 1) and use the solutions as the test solution and the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, cyclohexane, diethylamine (40 : 15 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly on the plate with a solution of sulfuric acid in methanol (2 in 5): the R_f value and color of the principal spot obtained from the test solution correspond to those obtained from the standard solution.

Dissolution Test Perform the test with 1 tablet of Fluphenazine Hydrochloride Tablets at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of 0.01 mol/L hydrochloric acid VS as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and perform the Assay with following differences: dilute the amount of the dissolved solution to be withdrawn with an equal volume of mobile phase without triethylamine; inject a volume of 100 μL, in the preparation of the standard solution, use a concentration and composition similar to that of the test solution; in the Mobile phase, use 0.3 % triethylamine; in the chromatographic system, use a flow rate of about 2.0 mL per minute.
The dissolution rate of Fluphenazine Hydrochloride Tablets in 45 minutes should be not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Fluphenazine Hydrochloride Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 mg of Fluphenazine Hydrochloride, to a separator, add 80 mL of mobile phase without triethylamine, shake well for 1 hour. Then sonicate for 10 minutes to make a fine colloid solution, add the mobile phase without triethylamine to make exactly 100 mL, and shake. Filter this solution, and use the filterate as the test solution. Separately, weigh accurately about 6 mg of Fluphenazine Hydrochloride RS, dissolve in the mobile phase without triethylamine to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under the Liquid Chromatographic according to the following conditions and, calculate the peak areas of Fluphenazine, A_T and A_S, for the test solution and the standard solution, respectively.

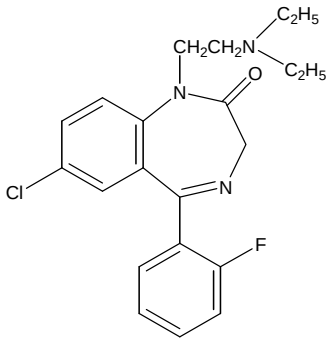
$$\text{Amount (mg) of fluphenazine } (C_{22}H_{26}F_3N_3OS \cdot 2HCl) = \text{Amount (mg) of Fluphenazine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector, column, column temperature, mobile phase and system suitability: Proceed as directed in the operating conditions in the Assay under Fluphenazine Hydrochloride.

Containers and Storage ***Containers***—Tight containers.

Flurazepam



$C_{21}H_{23}ClFN_3O$: 387.88

7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one [*17617-23-1*]

Flurazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of flurazepam ($C_{21}H_{23}ClFN_3O$).

Description Flurazepam appears as white to pale yellow crystals or crystalline powder.

Flurazepam is very soluble in chlorform, freely soluble in methanol, in ethanol (95), in acetic anhydride, or in ether and practically insoluble in water.

Identification (1) Dissolve 10 mg of Flurazepam in 3 mL of sulfuric acid: the solution shows a greenish yellow flurescence under ultraviolet light (main wavelength: 365 nm).

(2) Dissolve 10 mg of Flurazepam in 3 mL of citric acid · acetic acid TS and heat in a water-bath for 4 minutes: a dark red color develops.

(3) Prepare the test solution with 10 mg of Flurazepam as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests (2) for fluoride.

(4) Determine the absorption spectra of the solutions of Flurazepam and Flurazepam RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. And determine the spectra of the solutions of Flurazepam and Flurazepam RS in methanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths

(5) Perform the test with Flurazepam as directed under the Flame Coloration Test (2): a green color appears.

Melting Point 79 ~ 83 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Flurazepam in 10 mL of ethanol (95): the solution is clear and colorless to pale yellow.

(2) ***Chloride*** —Dissolve 1.0 g of Flurazepam in 50 mL of ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, separate the water layer, wash with two 20-mL volumes of ether and filter the water layer. Neutralize 20 mL of the filtrate with dilute nitric acid and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036 %)

(3) ***Sulfate***—Neurtralize 20 mL of the filtrate obtained in (2) with dilute hydrochloric acid and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Flurazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Flurazepam according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.20 g of Flurazepam in 20 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 20 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with flurescence indicator for Thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60 : 40 : 1) to a distance of about 12 cm and air-dry the plate. Examine under the ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, in vacuum, 60 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately 0.3 g of Flurazepam, previously dried, dissolve in 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS to the second equivalence point (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.394 mg of $C_{21}H_{23}ClFN_3O$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Flurazepam Capsules

Flurazepam Capsules contain not less than 93.0 % and not more than 107.0 % of the labeled amount of flurazepam ($C_{21}H_{23}ClFN_3O$: 387.88).

Method of Preparation Prepare as directed under Capsules, with Flurazepam.

Identification (1) Powder the contents of Flurazepam Capsules. To a quantity of the powder, equivalent to 0.1 g of Flurazepam according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir and filter. To 40 mL of the filtrate, add 80 mL of a solution of sodium hydroxide (1 in 250) and 100 mL of hexane, extract by shaking well and use the hexane layer as the test solution. Evaporate 25 mL of the test solution on a water-bath to dryness. Dissolve the residue in 3 mL of sulfuric acid: the solution shows a greenish yellow fluorescence under ultraviolet light.

(2) Evaporate 25 mL of the test solution obtained in (1) on a water-bath to dryness. Dissolve the residue in

3 mL of citric acid-acetic acid TS and heat in a water-bath for 4 minutes: a dark red color develops.

(3) Determine the absorption spectrum of the test solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 315 nm and 319 nm and a minimum between 297 nm and 301 nm.

Disintegration Test It meets the requirement.

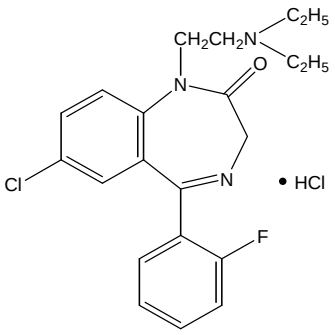
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 20 Flurazepam Capsules and powder the combined contents. Weigh accurately a portion of the powder, equivalent to about 50 mg of flurazepam ($C_{21}H_{23}ClFN_3O$), add 30 mL of methanol, stir well for 10 minutes and add methanol to make exactly 50 mL. Filter this solution, discard the first 20 mL of the filtrate, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Flurazepam RS, previously dried in vacuum at 60 ºC for 2 hours and dissolve in methanol to make exactly 50 mL. Pipet 6 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of test solution and the standard solution at 317 nm as directed under Ultraviolet-visible Spectrophotometry, respectively.

$$\text{Amount (mg) of flurazepam } (C_{21}H_{23}ClFN_3O) = \text{Amount (mg) of Flurazepam RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Flurazepam Hydrochloride



$C_{21}H_{23}ClFN_3O \cdot HCl$: 424.34

7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one hydrochloride [*36105-20-1*]

Flurazepam Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of flurazepam hydrochloride ($C_{21}H_{23}ClFN_3O \cdot HCl$).

Description Flurazepam Hydrochloride appears as white to yellowish white crystals or crystalline powder. Flurazepam Hydrochloride is freely soluble in water, in ethanol (95), in ethanol (99.5), or in acetic acid (100).

Melting point—About 197 ºC (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Flurazepam Hydrochloride and Flurazepam Hydrochloride RS in sulfuric acid · ethanol TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Flurazepam Hydrochloride and Flurazepam Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Flurazepam Hydrochloride (1 in 20) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Flurazepam Hydrochloride in 20 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Flurazepam Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Sulfate***—Perform the test with 1.5 g of Flurazepam Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Flurazepam Hydrochloride in a platinum crucible according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 50 mg of Flurazepam Hydrochloride in 5 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of this solution, and add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with the test and standard solutions as directed under the Thin-layer chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for Thin-layer chromatography. Place the plate in a chamber filled with ammonia vapor, allow to stand for about 15 minutes and immediately develop the plate with a mixture of ether and diethylamine (39 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 3 spots other than the principal spot and the spot on the starting point from the test solution appear and are not more intense than the spot from the

standard solution

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

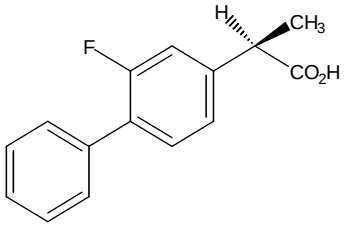
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Flurazepam Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.217 mg of $C_{21}H_{23}ClFN_3O \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Flurbiprofen



and enantiomer

$C_{15}H_{13}FO_2$: 244.26

(*RS*)-2-(2-fluorobiphenyl-4-yl)propanoic acid
[*5104-49-4*]

Flurbiprofen, when dried, contains not less than 98.0 % and not more than 101.0 % of flurbiprofen ($C_{15}H_{13}FO_2$).

Description Flurbiprofen is a white, crystalline powder and has a slightly irritating odor.
Flurbiprofen is freely soluble in methanol, in ethanol (95), in acetone or in ether, soluble in acetonitrile and practically insoluble in water.
A solution of Flurbiprofen in ethanol (95) (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectra of the solutions of Flurbiprofen and Flurbiprofen in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Flurbiprofen and Flurbiprofen RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 114 ~ 117 ºC.

Purity (1) ***Chloride***—Dissolve 0.6 g of Flurbiprofen in 40 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS, add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015 %).

(2) ***Heavy metals***—Dissolve 2.0 g of Flurbiprofen in 30 mL of acetone and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 30 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) ***Related substances***—Dissolve 20 mg of Flurbiprofen in 10 mL of a mixture of water and acetonitrile (11 : 9) and use this solution as the test solution. Pipet 1 mL of the test solution and add a mixture of water and acetonitrile (11:9) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: each area of the peaks other than the peak of flurbiprofen from the test solution is not larger than the peak area of flurbiprofen from the standard solution and the total area of these peaks is not larger than twice the peak area of Flurbiprofen from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 30 ºC.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (12 : 7 : 1).

Flow rate: Adjust the flow rate so that the retention time of Flurbiprofen is about 20 minutes.

System suitability

Test for required detectability: To exactly 5 mL of the standard solution, add a mixture of water and acetonitrile (11:9) to make exactly 25 mL. Confirm that the peak area of flurbiprofen from 20 μL of this solution is equivalent to 16 to 24 % of that of flurbiprofen from 20 μL of the standard solution.

System performance: Dissolve 0.04 g of flurbiprofen and 0.02 g of butyl parahydroxybenzoate in 100 mL of a mixture of water and acetonitrile (11:9). To 5 mL of this solution, add a mixture of water and acetonitrile (11:9) to make 50 mL. When the procedure is run with 20 μL of this solution under the above op-

erating conditions, butyl parahydroxybenzoate and flurbiprofen are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flurbiprofen is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of Flurbiprofen.

Loss on Drying Not more than 0.1 % (1 g, in vacuum at a pressure not exceeding 0.67 kPa, silica gel, 4 hours).

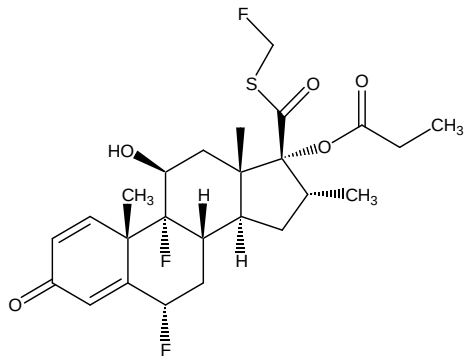
Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.6 g of Flurbiprofen, previously dried, dissolve in 50 mL of ethanol (95) and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $C_{15}H_{13}FO_2$

Containers and Storage ***Containers***—Well-closed containers.

Fluticasone Propionate



$C_{25}H_{31}F_3O_5S$: 500.57

(6*S*,8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-6,9-Difluoro-17-(((fluoromethyl)thio)carbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl propanoate [*80474-14-2*]

Fluticasone Propionate contains not less than 98.0 % and not more than 100.5 % of fluticasone propionate ($C_{25}H_{31}F_3O_5S$), calculated on the anhydrous and sovent-free basis.

Description Fluticasone Propionate is as a white, fine powder.
Fluticasone Propionate is sparingly soluble in dichloromethane, slightly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the infrared spectra of Fluticasone Propionate and Fluticasone Propionate RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak from the test solution is same as that from the standard solution obtained in the Assay.

Specific Optical Rotation $[\alpha]_D^{20}$: +32 ~ +36° (calculated on the anhydrous and solvent-free basis, 0.1 g, dichloromethane, 20 mL, 100 mm).

Purity (1) ***Related substances***—Dissolve 20 mg of Fluticasone Propionate in 5.0 mL of the mobile phase A by sonication, add 5.0 mL of the mobile phase C and use this solution as the test solution. Perform the test with 50 μL of the test solution as directed in the area percentage method under Liquid Chromatography according to the following conditions, and measure the area of each realted substance and the total area of all of the related substances, A_i and A_S: the limit of the related substances are as shown in Table I.

Table 1

Related substances	Relative retention time	Limit (%)
Fluticasone propionate related substance I	0.5	0.2
Fluticasone propionate related substance II	0.75	0.1
Fluticasone propionate related substance III	0.8	0.1
Fluticasone propionate related substance IV	0.95	0.3
Fluticasone propionate	1.0	-
Fluticasone propionate related substance V	1.3	0.3
Other related substances	-	0.1
Total related substances	-	1.0

Any related substances of less than 0.05 % are disregarded from the total amount of related substances.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 239 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed

with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

Mobile phase A: Mix 0.5 mL of phosphoric acid with 1000 mL of acetonitrile

Mobile phase B: Mix 0.5 mL of phosphoric acid with 1000 mL of methanol

Mobile phase C: Mix 0.5 mL of phosphoric acid with 1000 mL of water

Table 2

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)	Mobile phase C (vol %)
0	42	3	55
0-40	42→53	3	55→44
40-60	53→47	3	44→10
60-70	87	3	10
70-75	87→42	3	10→55

Flow rate : 1.5 mL/minute

System suitability

System performance: Weigh about 2.0 mg of fluticasone propionate in 5.0 mL of the mobile phase A by sonication, add 5.0 mL of the mobile phase C and use this solution as the system suitability solution. When the procedure is performed with 50 µL of this solution accoding to the above operating conditions, the resolution between peaks of fluticasone propionate related substance II and fluticasone propionate related substance III is not less than 1.5 and their relative retion times are as shown in Table I.

(2) ***Bromofluoromethane***—Dissolve 0.2 g of Fluticasone Propionate in 1 mL of *N,N*-dimethylformamide and use this solution as the test solution. Separately, measure 20 µL of bromofluoromethane, add *N,N*-dimethyl-formamide to make 10 mL, take 10 µL of this solution, and add *N,N*-dimethylformamide to make 1 mL. Take 10 µL of this solution, add *N,N*-dimethylformamide to make 1 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and determine heights of peaks of bromofluoromethane: the height of the bromofluoromethane obtained from the test solution is less than that from the standard solution.

Operating conditions

Detector : An electron capture detector.

Column : A capillary column, about 0.32 mm in internal diameter and about 25 m in length, coated with 5 % phenyl-95 % methylpolysiloxane with the thickness of 5 µm.

Column temperature: Initially the temperature of the column is maintained at 40 ºC for 3.5 minutes, then the temperature is increased at a rate of 30 ºC per minute to 200 ºC, and maintained at 200 ºC for 10 minutes.

Carrier gas: Nitrogen

Flow rate: 2.8 mL/minute

Inlet temperature: 85 ºC

Split ratio: about 70 : 1

Detector temperature: 250 ºC

(3) ***Acetone***—Dissolve 0.5 g of Fluticasone Propionate, accurately weighed, in the internal standard solution to make exactly 10 mL and use this solution as the test solution. Separately, take exactly 50 µL of acetone, add the internal standard solution to make exactly 100 mL and use this solution as the standard solution. Perform the test with 0.1 µL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of acetone to that of the internal standard, for the test solution and the standard solution, respectively (not more than 1.0 %).

$$\text{Amount (\%) of acetone} = 0.05 \times \frac{D}{C} \times \frac{Q_T}{Q_S}$$

D: Density of acetone at 20 ºC

C: Concentration (g/mL) of Fluticasone Propionate in the test solution

Internal standard solution—To 50 µL of tetrahydrofuran, add *N,N*-dimethylformamide to make 100 mL.

Operating conditions

Detector : A hydrogen flame ionization detector.

Column : A capillary column, about 0.53 mm in internal diameter and about 25 m in length, the inside coated with polyethylene glycol (mean molecular mass is between 3000 and 3700) at the thickness of 2 µm.

Column temperature: Initially the temperature of the column is maintained at 60 ºC for 3.5 minutes, then the temperature is increased at a rate of 30 ºC per minute to 180 ºC, and maintained at 180 ºC for 3 minutes.

Carrier gas: Nitrogen or helium

Flow rate: 5.5 mL/minute

Inlet temperature: 150 ºC

Detector temperature: 250 ºC

System suitability

System repeatability: When the test is repeated 6 times with 0.1 µL each of the standard solution under the above operating conditions, the relative standard deviation of the peak height of acetone is not more than 5.0 %.

Water Not more than 0.2 % (1 g, volumetric titration, direct titration).

Assay Dissolve about 40 mg each of Fluticasone Propionate and Fluticasone Propionate RS, accurately weighed, in the mobile phase to make exactly 100 mL. Pipet 5.0 mL of this solution and add the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and measure the areas of fluticasone propionate obtained from each solutions, A_T and A_S.

$$\text{Amount (mg) of fluticasone propionate } (C_{25}H_{31}F_3O_5S) = \text{Amount (mg) of Fluticasone Propionate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 239 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of methanol, 0.01 mol/L ammonium dihydrogen phosphate buffer, and acetonitrile (50 : 35 : 15).

Flow rate: 1.5 mL/minute.

System suitability

System performance: Dissolve 2.0 mg of fluticasone propionate related substance IV RS in 50 mL of the mobile phase. When the procedure is run with 20 μL each of this solution and the standard solution under the above operating conditions, relative retention times peaks of fluticasone propionate related substance IV and fluticasone propionate are 1.1 and 1.0, respectively, and the resolution between them is not less than 1.5.

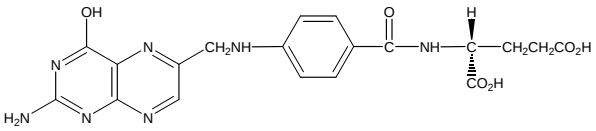
System reapetibility: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluticasone propionate is not more than 2.0 %.

0.01 mol/L ammonium dihydrogen phosphate buffer—Dissolve 11.5 g of ammonium dihydrogen phosphate in 1000 mL of water and adjust to a pH of 3.5 ± 0.05 with phosphoric acid.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 30 °C.

Folic Acid



$C_{19}H_{19}N_7O_6$: 441.40

(2*S*)-2-[(4-{[(2-Amino-4-hydroxypteridin-6-yl)methyl]amino}phenyl)formamido]pentanedioic acid [*59-30-3*]

Folic Acid, when dried, contains not less than 98.0 % and not more than 102.0 % of folic acid ($C_{19}H_{19}N_7O_6$).

Description Folic Acid is a yellow to orange, crystalline powder and is odorless.

Folic Acid is practically insoluble in water, in methanol, in ethanol (95), in pyridine or in ether.

Folic Acid dissolves in hydrochloric acid, in sulfuric acid, in dilute sodium hydroxide TS or in a solution of sodium carbonate (1 in 100) and these solutions are yellow in color.

Folic Acid is affected gradually by light.

Identification (1) Dissolve 1.5 mg each of Folic Acid and Folic Acid RS in dilute sodium hydroxide TS to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Take 10 mL of the solution obtained with Folic Acid in (1), add 1 drop of potassium permanganate TS and mix well until the color changes to blue and immediately observe under ultraviolet light (main wavelength: 365 nm): a blue fluorescence is produced.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Folic Acid in 10 mL of dilute sodium hydroxide TS: the solution is clear and yellow in color.

(2) ***Free amines***—Pipet 30 mL of the test solution obtained in the Assay, add 20 mL of dilute hydrochloric acid and water to make accurately 100 mL and use this solution as the test solution. Weigh accurately about 50 mg of *p*-aminobenzoylglutamic acid RS, previously dried in a desicator (in vacuum, silica gel) for 4 hours, dissolve in diluted ethanol (2 in 5) to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 1000 mL and use this solution as the standard solution. Pipet 4 mL each of the test solution and the standard solution, proceed as directed in the Assay and perform the test as directed under Ultraviolet-visible Spectrophotometry. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 550 nm: the content of free amines is not more than 1.0 %.

$$\text{Content (\%) of free amines} = \frac{A_T}{A_S} \times \frac{W'}{W}$$

W: Amount (mg) of Folic Acid, calculated on the anhydrous basis,

W': Amount (mg) of *p*-aminobenzoyl-glutamic acid RS.

Water Not more than 8.5 % (10 mg, coulometric titration).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Weigh accurately about 50 mg each of Folic Acid and Folic Acid RS. To each, add 50 mL of dilute sodium hydroxide TS, mix well to dissolve, add dilute sodium hydroxide TS to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. To 30 mL each of the test solution and the standard solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Take 60 mL each of these solutions, add 0.5 g of zinc powder and allow to stand with frequent shaking for 20 minutes. Filter each mixture through a dry filter paper and discard the first 10 mL of the filtrate. Pipet 10 mL each of the subsequent filtrate and add water to make exactly 100 mL. To 4 mL each of solutions, accurately measured, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of a solution of sodium nitrite (1 in 1000), mix well and allow to stand for 2 minutes. To each solution, add 1 mL of a solution of ammonium sulfate (1 in 200), mix thoroughly and allow to stand for 2 minutes. To each of these solution, add 1 mL of a solution of *N*-(1-naphthyl)-*N'*-diethylethlyen ediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes and add water to make exactly 20 mL. Separately, to 30 mL of the test solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution and add 18 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 4 mL of this solution and prepare the blank solution in the same manner as the test solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 4 mL of water in the same manner as a blank. Determine the absorbances, A_T, A_S and A_C, of the test solution, the standard solution and the blank solution, respectively, at 550 nm.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = \text{Amount (mg) of Folic Acid RS, calculated on the anhydrous basis} \times \frac{A_T - A_C}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Folic Acid Tablets

Folic Acid Tablets contain not less than 90.0 % and not more than 115.0 % of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$: 441.40).

Method of Preparation Prepare as directed under Tablets, with Folic Acid.

Identification (1) Take a portion of powdered Folic Acid Tablets, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add 100 mL of dilute sodium hydroxide TS, shake well and filter. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the test solution and perform the test as directed in the Identification (2) under Folic Acid.

(2) Determine the absorption spectrum of the solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm, and between 361 nm and 369 nm and when A_1 is the absorbance at the absorption maximum between 255 nm and 257 nm, and A_2 is the absorbance at the absorption maximum between 361 nm and 369 nm, the ratio, A_2/A_1, is between 2.80 and 3.00.

Dissolution Test Perform the test with 1 tablet of Folic Acid Tablets at 50 revolutions per minute according to Method 2, using 500 mL of water as the dissolution solution. Take the dissolved solution 45 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the diluent to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Folic Acid RS (previously determine the water), dissolve in the diluent to make the same concentration as the test solution, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of folic acid in each solution. The dissolution rate of Folic Acid Tablets in 45 minutes is not less than 75 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount of folic acid } (C_{19}H_{19}N_7O_6) = C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 50000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of folic acid ($C_{19}H_{19}N_7O_6$) in 1 tablet

Diluent—To 2 mL of ammonium hydroxide and 1 g of sodium perchlorate add water to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: To 35.1 g of sodium perchlorate and 1.40 g of potassium dihydrogen phosphate add 7.0 mL of 1 mol/L potassium hydroxide, 40 mL of methanol, and water to make 1000 mL. Adjust the pH to 7.2 with 1 mol/L potassium hydroxide or phosphoric acid.

Flow rate: 1 mL/minute

System suitability

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of folic acid is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

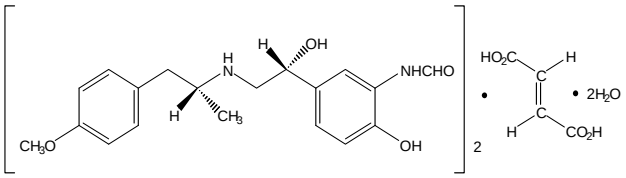
Assay Weigh accurately and powder not less than 20 Folic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$: 441.40). Add 50 mL of dilute sodium hydroxide TS, shake frequently, then filter into a volumetric flask and wash with dilute sodium hydroxide TS. Take the combined filtrate and washings, add dilute sodium hydroxide TS to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Folic Acid RS, dissolve in dilute sodium hydroxide TS to make exactly 100 mL and use this solution as the standard solution. Take 30 mL each of the test solution and the standard solution, exactly measured and proceed as directed in the Assay under Folic Acid.

$$\text{Amount (mg) of Folic Acid } (C_{19}H_{19}N_7O_6) = \text{Amount (mg) of Folic Acid RS, calculated on the anhydrous basis} \times \frac{A_T - A_C}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Formoterol Fumarate Hydrate



$(C_{19}H_{24}N_2O_4)_2C_4H_4O_4 \cdot 2H_2O$: 840. 91

N-{2-Hydroxy-5-{(*RS*)-1-hydroxy-2-{[(*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino}ethyl}phenyl} formamide (*E*)-but-2-enedioate [*43229-80-7,* anhydride]

Formoterol Fumarate Hydrate contains not less than 98.5 % and not more than 101.0 % of formoterol fumarate $[(C_{19}H_{24}N_2O_4)_2C_4H_4O_4$: 804.882], calculated on the anhydrous basis.

Description Formoterol Fumarate Hydrate is a white to yellowish white, crystalline powder.

Formoterol Fumarate Hydrate is freely soluble in acetic acid (100), soluble in methanol, very slightly soluble in water or in ethanol (95) and practically insoluble in ether.

A solution of Formoterol Fumarate in methanol (1 in 100) shows no optical rotation.

Meting point—About 138 ºC (with decomposition).

Identification (1) Dissolve 0.5 g of Formoterol Fumarate Hydrate in 20 mL of 0.5 mol/L sulfuric acid TS and extract with three 25 mL volumes of ether. Wash the combined ether extracts with 10 mL of 0.5 mol/L sulfuric acid TS and evaporate the ether layer under reduced pressure and dry the residue at 105 ºC for 3 hours: the residue melts at about 290 ºC (with decomposition, in a sealed tube).

(2) Determine the absorption spectra of solutions of Formoterol Fumarate Hydrate and Formoterol Fumarate Hydrate RS in methanol (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Formoterol Fumarate Hydrate and Formoterol Fumarate Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Formoterol Fumarate Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.20 g of Formoterol Fumarate Hydrate in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (20 : 20 : 10 : 3) to a distance of about 12 cm and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the test solution are not more intense than the spot from the

standard solution.

Water 4.0 ~ 5.0 % (0.5 g, volumetric titration, direct titration).

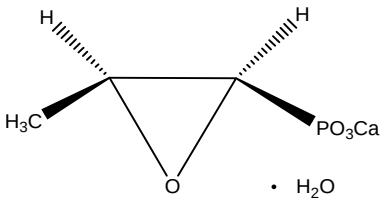
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.7 g of Formoterol Fumarate Hydrate, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.24 mg [$(C_{19}H_{24}N_2O_4)_2C_4H_4O_4$]

Containers and Storage ***Containers***—Tight containers.

Fosfomycin Calcium Hydrate



$C_3H_5CaO_4P \cdot H_2O$: 194.14

Calcium [(2*R*,3*S*)-3-methyloxiran-2-yl]phosphonate [*26016-98-8*]

Fosfomycin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.
Fosfomycin Calcium Hydrate contains not less than 725 μg (potency) and not more than 805 μg (potency) per mg of fosfomycin ($C_3H_7O_4P$: 138.06), calculated on the anhydrous basis.

Description Fosfomycin Calcium Hydrate appears as white crystalline powder.
Fosfomycin Calcium Hydrate is slightly soluble in water, and practically insoluble in methanol or in ethanol (99.5).

Identification (1) Determine the infrared spectra of Fosfomycin Calcium Hydrate and Fosfomycin Calcium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Fosfomycin Calcium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 300) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylpropansulfonate as an internal reference compound: it exhibits a double signal at around δ 1.5 ppm, a duple double signal at around δ 2.9 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.4 ppm.

(3) A solution of Fosfomycin Calcium Hydrate (1 in 500) responds to the Qualitative Tests (3) for calcium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -2.5 ~ -5.4° (0.5 g calculated on the anhydrous basis, 0.4 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5, 10 mL, 100 mm).

pH Weigh 40 mg of Fosfomycin Calcium Hydrate, suspend in 10 mL of water, allow to cool to about 5 ºC, and raise the temperature to room temperature. The pH of the susepension is between 8.0 and 10.0.

Purity (1) ***Heavy metals***—To 1.0 g of Fosfomycin Calcium Hydrate add 40 mL of 0.25 mol/L acetic acid solution and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Fosfomycin Calcium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

Water Not more than 12.0 % (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Calcium Hydrate, add exactly 40 mL of a solution of sodium periodate (107 in 10000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add exactly 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the test stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogen phosphate, proceed with this solution in the same manner as directed for the preparation of the test stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the test stock solution without using Fosfomycin Calcium Hydrate, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the test stock solution, standard stock solution, and blank stock solution, transfer to separate 25 mL volumetric flasks, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and use these solutions as the test solution, standard solution, and blank solution, respectively. After

allowing these solutions to stand at 20 ± 1 °C for 30 minutes, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using water as a blank, and determine the absorbances, A_T, A_S, and A_B, at 740 nm of the test solution, standard solution, and blank solution (not less than 15.2 % and not more than 16.7 %).

$$\text{Amount (mg) of phosphorus} = W \times \frac{A_T - A_B}{A_S - A_B} \times 0.22760$$

W: Amount (mg) of potassium dihydrogen phosphate taken

0.22760: Content of phosphorus in potassium dihydrogen phosphate

Calcium Content Weigh accurately about 0.2 g of Fosfomycin Calcium Hydrate, add exactly 4 mL of 1 mol/L hydrochloric acid TS, shake until completely dissolved, add exactly 100 mL of water, 9 mL of sodium hydroxide TS, and 0.1 g of methylthymol blue-sodium chloride indicator, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from clear blue to gray or gray-purple. Perform a blank determination and make any necessary correction (not less than 19.6 % and not more than 21.7 %).

Each mL of 0.05 mol/L
disodium dihydrogen ethylenediamine tetraacetate VS
= 2.004 mg of Ca

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the media (1) under the Assay in Fosfomycin Sodium.

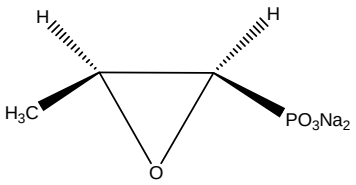
(2) Test organism- *Proteus* sp. MB 838.

(3) Test organism suspension- Prepare according to (3) under the Assay in Fosfomycin Sodium.

(4) Weigh accurately an amount of Fosfomycin Calcium Hydrate, equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L Tris buffer solution (pH 7.0) to make exactly 50 mL. Pipet a suitable volume of this solution, add 0.05 mol/L Tris buffer solution (pH 7.0) so that each mL contains 10.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Fosfomycin Phenethylammonium RS, dissolve in 0.05 mol/L Tris buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable volume of the standard stock solution, add 0.05 mol/L Tris buffer solution (pH 7.0) so that each mL contains 10.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Fosfomycin Sodium



$C_3H_5Na_2O_4P$: 182.02

Sodium [(2*R*,3*S*)-3-methyloxiran-2-yl]phosphonate
[*26016-99-9,* anhydride]

Fosfomycin Sodium is the sodium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.
Fosfomycin Sodium contains not less than 725 μg (potency) and not more than 770 μg (potency) per mg of fosfomycin ($C_3H_7O_4P$: 138.06), calculated on the anhydrous basis.

Description Fosfomycin Sodium appears as white crystalline powder.
Fosfomycin Sodium is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification (1) Determine the infrared spectra of Fosfomycin Sodium and Fosfomycin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Fosfomycin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 300) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylpropansulfonate as an internal reference compound: it exhibits a double signal at around δ 1.5 ppm, a duple double signal at around δ 2.8 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.3 ppm.

(3) A solution of Fosfomycin Sodium (1 in 500) responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -3.5 ~ -5.5° (calculated on the anhydrous basis, 0.5 g, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.70 g of Fosfomycin Sodium in 10 mL of water is between 8.5 and 10.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Fosfomycin Sodium in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Fosfomycin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Fosfomycin Sodium according to Method 3, and perform the test (not more than 2 ppm).

Water Not more than 3.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Fosfomycin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.125 EU/mg (potency) of fosfomycin, when Fosfomycin Sodium is used in a sterile preparation.

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Sodium, add exactly 40 mL of a solution of sodium periodate (107 in 10000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add exactly 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the test stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogen phosphate, proceed with this solution in the same manner as directed for the preparation of the test stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the test stock solution without using Fosfomycin Sodium, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the test stock solution, standard stock solution, and blank stock solution, transfer to separate 25 mL volumetric flasks, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and use these solutions as the test solution, standard solution, and blank solution, respectively. After allowing these solutions to stand at 20 ± 1 °C for 30 minutes, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using water as a blank, and determine the absorbances, A_T, A_S, and A_B, at 740 nm of the test solution, standard solution, and blank solution (not less than 16.2 % and not more than 17.9 %).

$$\text{Amount (mg) of phosphorus} = W \times \frac{A_T - A_B}{A_S - A_B} \times 0.22760$$

W: Amount (mg) of potassium dihydrogen phosphate taken

0.22760: Content of phosphorus in potassium dihydrogen phosphate

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer-

Peptone	5.0 g	Yeast extract	2.0 g
Meat extract	3.0 g	Agar	15 g
Water	1000 mL		

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be between 6.5 and 6.6 after sterilization.

(2) Test organism- *Proteus* sp. MB 838.

(3) Test organism suspension- Incubate the test organism on the slant of the agar medium for transferring test organism at 37 ºC for 40 to 48 hours. Sub-culture at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organism in a Roux bottle, incubate at 37 ºC for 40 to 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water to dilute 10-fold so that the percent transmission at 560 nm of the suspension is 17 %. Keep the suspension of test organism at 10 ºC or below and use within 7 days. Add 1 to 2 mL of the suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48 ºC, mix with shaking, and use this as the seeded agar layer.

(4) Weigh accurately an amount of Fosfomycin Sodium, equivalent to about 20 mg (potency), and add 0.05 mol/L Tris buffer solution (pH 7.0) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Fosfomycin RS, dissolve in 0.05 mol/L Tris buffer solution (pH 7.0) to make exactly 50 mL, and use the solution as the standard stock solution. Keep the standard stock solution below 5 °C, and use within 7 days. Take exactly a suitable amount of the standard stock solution, add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Hermetic containers.

Fosfomycin Sodium for Injection

Fosfomycin Sodium for Injection is a preparation for injection, which is dissolved before use.

Fosfomycin Sodium for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of fosfomycin ($C_3H_7O_4P$: 138.06).

Method of Preparation Prepare as directed under Injections, with Fosfomycin Sodium.

Description Fosfomycin Sodium for Injection appears as white crystalline powder.

Identification (1) Dissolve about 0.1 g of Fosfomycin Sodium for Injection in 3 mL of a solution of perchloric acid (1 in 4), add 1 mL of 0.1 mol/L sodium periodate solution, and warm in a water bath at 60 °C for 30 minutes. After cooling, add 50 mL of water, and neutralize with a saturated solution of sodium hydrogen carbonate. To this solution add 1 mL of potassium iodide TS: the solution does not reveal a red color, while the blank solution reveals a red color.

(2) To 2 mL of a solution of Fosfomycin Sodium for Injection (1 in 250) add 1 mL of perchloric acid and 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-5-sulfonic acid TS, and allow to stand for 30 minutes: a blue color develops.

(3) Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 0.1 g (potency) of fosfomycin sodium according to the labeled amount, in 50 mL of water: the solution responds to the Qualitative Tests (1) for sodium salt.

pH Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of fosfomycin sodium in 20 mL of water: the pH of this solution is between 6.5 and 8.5.

Purity ***Clarity and color of solution***—Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of fosfomycin sodium according to the labeled amount, in 10 mL of water: the solution is clear and colorless.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.025 EU/mg (potency) of fosfomycin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

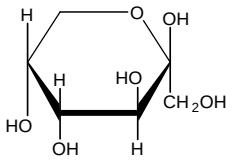
Uniformity of Dosage Units It meets the requirement.

Water Not more than 4.0 % (0.1 g, coulometric titration).

Assay Proceed as directed in the Assay under Fosfomycin Sodium. Weigh accurately the mass of the contents of not less than 10 containers of Fosfomycin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of fosfomycin sodium according to the labeled amount, and dissolve in 0.05 mol/L Tris buffer solution (pH 7.0) to make exactly 50 mL. Pipet a suitable volume of this solution, add 0.05 mol/L Tris buffer solution (pH 7.0) so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Fructose



$C_6H_{12}O_6$: 180.16

(3*S*,4*R*,5*R*)-1,3,4,5,6-Pentahydroxyhexan-2-one
[*57-48-7*]

Fructose, when dried, contains not less than 98.0 % and not more than 101.0 % of fructose ($C_6H_{12}O_6$).

Description Fructose appears as colorless to white crystals or crystalline powder, is odorless and has a sweet taste.
Fructose is very soluble in water, sparingly soluble in ethanol (95) and practically insoluble in ether.
Fructose is hygroscopic.

Identification (1) Add 2 to 3 drops of a solution of Fructose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) Determine the infrared spectra of Fructose and Fructose RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 4.0 g of Fructose in 20 mL of water: the pH of the solution is between 4.0 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 25.0 g of Fructose in 50 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—To a mixture of 3 mL of iron (III)

chloride hexahydrate stock CS, 1 mL of cobalt (II) chloride hexahydrate stock CS and 2.0 mL of cupric sulfate stock CS, add water to make 10.0 mL. To 3.0 mL of the solution, add water to make 50 mL.

(2) ***Acid***—Dissolve 5.0 g of Fructose in 50 mL of freshly boiled and cooled water and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color is observed.

(3) ***Chloride***—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(4) ***Sulfate***—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(5) ***Sulfite***—Dissolve 0.5 g of Fructose in 5 mL of water and add 0.25 mL of 0.01 mol/L iodine: the color of the solution is yellow.

(6) ***Heavy metals***—Proceed with 5.0 g of Fructose according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 4 ppm).

(7) ***Calcium***—Dissolve 0.5 g of Fructose in 5 mL of water, add 2 to 3 drops of ammonia TS and 1 mL of ammonium oxalate TS and allow to stand for 1 minute: the solution is clear.

(8) ***Arsenic***—Dissolve 1.5 g of Fructose in 5 mL of water, heat with 5 mL of dilute sulfuric acid and 1 mL of bromine TS on a water-bath for 5 minutes, concentrate to 5 mL and cool. Perform the test with this solution as the test solution (not more than 1.3 ppm).

(9) ***5-Hydroxymethylfurfurals***—Dissolve 5.0 g of Fructose in 100 mL of water and read the absorbance at 284 nm as directed under Ultraviolet-visible Spectrophotometry: the absorbance is not more than 0.32.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 4 g of Fructose, previously dried, dissolve in 0.2 mL of ammonia TS and 80 mL of water and after standing for 30 minutes, add water to make exactly 100 mL and determine the optical rotation, α_D, in a 100 mm cell at 20 ± 1 ºC as directed under the Optical Rotation Determination.

$$\text{Amount (mg) of fructose } (C_6H_{12}O_6) = |\alpha_D| \times 1087.0$$

Containers and Storage ***Containers***—Tight containers.

Fructose Injection

Fructose Injection is an aqueous solution for injection. Fructose Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of fructose ($C_6H_{12}O_6$: 180.16).

Method of Preparation Prepare as directed under Injections, with Fructose. No preservative is added.

Description Fructose appears as colorless to white crystals or crystalline powder, is odorless and has a sweet taste.

Identification (1) Take a volume of Fructose Injection, equivalent to 1 g of Fructose according to the labeled amount, dilute with water or concentrate on a water-bath to make 20 mL, if necessary and use this solution as the test solution. Add 2 to 3 drops of the test solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) Take 10 mL of the test solution obtained in (1), add 0.1 g of resorcin and 1 mL of hydrochloric acid and warm on a water-bath for 3 minutes: a red color is observed.

pH 3.0 ~ 6.5. When the labeled concentration exceeds 5 %, prepare a 5 % solution with water before the test.

Purity (1) ***Heavy metals***—Take a volume of Fructose Injection, equivalent to 5.0 g of Fructose and evaporate on a water-bath to dryness. Proceed with the residue according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution.

(2) ***Arsenic***—Take a volume of Fructose Injection, equivalent to 1.5 g of Fructose according to the labeled amount, dilute with water or concentrate on a water-bath to make 5 mL, if necessary and add 5 mL of dilute sulfuric acid and 1 mL of bromine TS. Proceed as directed in the Purity (8) under Fructose.

(3) ***5-Hydroxymethylfurfurals***—Take an amount of Fructose Injection equivalent to 5.0 g, evaporate or add water to make 100 mL and read the absorbance at 284 nm as directed under Ultraviolet-visible Spectrophotometry: the absorbance is not more than 0.32.

Residue on Ignition Measure exactly a volume of Fructose Injection, equivalent to about 2.0 g of Fructose according to the labeled amount, evaporate on a water-bath to dryness and perform the test: the residue weighs not more than 2.0 mg.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mL of Fructose Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

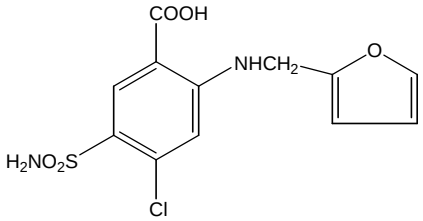
Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Fructose Injection, equivalent to about 4 g of fructose ($C_6H_{12}O_6$: 180.16), add 0.2 mL of ammonia TS, dilute with water to make exactly 100 mL, shake well and after allowing to stand for 30 minutes, determine the optical rotation, α_D, in a 100 mm cell at 20 ± 1 ºC as directed under the Optical Rotation Determination.

Amount (mg) of fructose ($C_6H_{12}O_6$) = $|\alpha_D| \times 1087.0$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Furosemide



$C_{12}H_{11}ClN_2O_5S$: 330.74

4-Chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid [*54-31-9*]

Furosemide, when dried, contains not less than 98.0 % and not more than 101.0 % of furosemide ($C_{12}H_{11}ClN_2O_5S$).

Description Furosemide appears as white crystals or crystalline powder.
Furosemide is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetonitrile or in acetic acid (100) and practically insoluble in water.
Furosemide dissolves in dilute sodium hydroxide TS.
Furosemide is gradually colored by light.
Melting point—About 205 ºC (with decomposition).

Identification (1) Dissolve 25 mg of Furosemide in 10 mL of methanol. To 1 mL of this solution, add 10 mL of 2 mol/L hydrochloric acid TS. Heat the solution in a water-bath under a reflux condenser for 15 minutes, cool and add 18 mL of sodium hydroxide TS to make weakly acidic: this solution responds to the Qualitative Tests for primary aromatic amines. A red to red-purple color is observed.

(2) Determine the absorption spectra of solutions of Furosemide and Furosemide RS in dilute sodium hydroxide TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Furosemide and Furosemide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Furosemide in 10 mL of a solution of sodium hydroxide (1 in 50): the solution is colorless and clear.

(2) ***Chloride***—Dissolve 2.6 g of Furosemide in 90 mL of dilute sodium hydroxide TS, add 2 mL of nitric acid and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.020 %).

(3) ***Sulfate***—To 20 mL of the filtrate in (2), add 1 mL of dilute hydrochloric acid and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of hydrochloric acid and water to make 50 mL (not more than 0.030 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Furosemide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Related substances***— Dissolve 25 mg of Furosemide in 25 ml. of the dissolving solution, and use this solution as the test solution. Pipet 1 mL of the test solution, add the dissolving solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak appeared ahead of the peak of furosemide is not more than 2/5 times the peak area of furosemide from the standard solution, the area of each peak appeared behind the peak of furosemide is not more than 1/4 times the peak area of furosemide from the standard solution, and the total area of these peaks is not more than 2 times the peak area of furosemide from the standard solution.

Dissolving solution—To 22 mL of acetic acid (100), add a mixture of water and acetonitrile (1 : 1) to make l000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water, tetrahydrofuran and acetic acid (100) (70 : 30 : 1).

Flow rate: Adjust the flow rate so that the retention time of furosemide is about 18 minutes.

System suitability

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the dissolving solution to make exactly 50 mL. Confirm that the peak area of furosemide obtained from 20 μL of this solution is equivalent to 3.2 to 4.8 % of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetrry factor of the peak of furosemide are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of furosemide is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention rime of furosemide beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Furosemide, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination with a mixture of 50 mL of *N,N*-dimethylformamide and 15 mL of water and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.074 mg of $C_{12}H_{11}ClN_2O_5S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Furosemide Tablets

Furosemide Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$: 330.74).

Method of Preperation Prepare as directed under Tablets, with Furosemide.

Identification (1) Shake well a quantity of powdered Furosemide Tablets, equivalent to 0.2 g of furosemide according to the labeled amount, with 40 mL of acetone, and filter. To 0.5 mL of the filtrate add 10 ml of 2 mol/L hydrochloric acid TS and heat under a reflux condenser on a water bath for 15 minutes. After cooling, add 18 mL of sodium hydroxide TS to make the solution slightly acidic: the solution responds to the Qualitative Tests for Primary Aromatic Amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of the test solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry : it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nn, and between 330 nm and 336 nm.

Purity To a quantiy of powdered Furosemide Tablets, equivalent to 40 mg of furosemide according to the labeled amount, add about 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge the solution, add 3.0 mL of water to 1.0 mL of the clear supernatant liquid, cool in ice, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Add 1.0 ml. of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of *N,N*-diethyl-*N'*-1-naphthyl-ethylenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spcctrophotometry, using a solution prepared in the same manner with 1.0 mL of acetone as the blank: the absorbance at 530 nm is not more than 0.10.

Dissolution Test Perform the test with 1 tablet of Furosemide Tablets at 50 revolutions per minute according to the Method 2, using 900 mL of 2nd fluid for dissolution test as the dissolution solution. Withdraw 20 mL or more of the dissolved solution 15 minutes after starting the test for a 20-mg tablet or 30 minutes after for a 40-mg tablet, and filter through a membrane filter with a pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about l0 μg of furosemide ($C_{12}H_{11}ClN_2OS$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105 °C for 4 hours, and dissolve in 5 mL of methanol, and add 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S of the test solution and the standard solution at 277 nm as directed under Ultraviolet-visible Spectrophotometry.

The dissolution rate of Furosemide Tablets in 60 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$)

$$= \text{Amount (mg) of Furosemide RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 45$$

C: Labeled amount (mg) of furosemide ($C_{12}H_{11}ClN_2O_5S$) in 1 tablet

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement.

To 1 tablet of Furosemide Tablets add a suitable amount of 0.05 mol/L sodium hydroxide TS, shake to disintegrate, then add 0.05 mol/L sodium hydroxide TS to make exactly *V* mL, so that each mL contains about 0.4 mg of furosemide ($C_{12}H_{11}ClN_2O_5S$). Filter the solution, discard the first 10 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the test solution. Proceed as directed in the Assay.

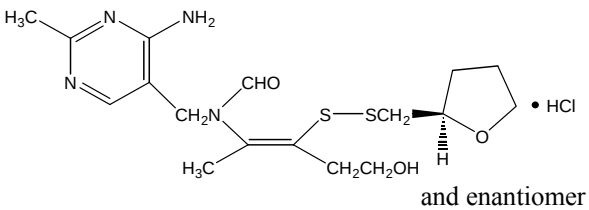
$$\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) = \text{Amount (mg) of Furosemide RS} \times \frac{A_T}{A_S} \times \frac{V}{50}$$

Assay Weigh accurately the mass of not less than 20 Furosemide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of furoscmide ($C_{12}H_{11}ClN_2O_5S$), add about 70 mL of 0.05 mol/L sodium hydroxide TS, shake well, and add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105 °C for 4 hours, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.05 mol/l. sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 271 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) = \text{Amount (mg) of Furosemide RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Fursultiamine Hydrochloride



and enantiomer

$C_{17}H_{26}N_4O_3S_2 \cdot HCl$: 435.00

N-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-*N*-{(1*E*)-4-hydroxy-1-methyl-2-[((2*R*)-tetrahydrofuran-2-ylmethyl)disulfanyl]but-1-en-1-yl}formamide hydrochloride [*2105-43-3*]

Fursultiamine Hydrochloride contains not less than 98.5 % and not more than 101.0 % of fursultiamine hydrochloride ($C_{17}H_{26}N_4O_3S_2 \cdot HCl$), calculated on the anhydroys basis.

Description Fursultiamine Hydrochloride appears as white crystals or crystalline powder, is odorless or has a characteristic odor and has a bitter taste.

Fursultiamine Hydrochloride is freely soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.

Identification (1) Dissolve 5 mg of Fursultiamine Hydrochloride in 6 mL of 0.1 mol/L hydrochloric acid TS, add 0.1 g of zinc dust, allow to stand for several minutes and filter. To 3 mL of the filtrate, add 3 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS, then add 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, allow to stand to separate the isobutanol layer and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. The fluorescence disappears by acidifying and appears again by alkalifying.

(2) Determine the infrared spectra of Fursultiamine Hydrochloride and Fursultiamine Hydrochloride RS, previously dried in a desiccator (in vacuum, P_2O_5) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any differences appear, dissolve the Fursultiamine Hydrochloride in water, evaporate the water and dry the residue in a desiccator (in vacuum, P_2O_5) for 24 hours and repeat the test.

(3) A solution of Fursultiamine Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Fursultiamine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 1.5 g of Fursultiamine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS: not more than 0.011 %.

(3) ***Heavy metals***—Proceed with 1.0 g of Fursultiamine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Fursultiamine Hydrochloride in 100 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard

solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Determine peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of fursultiamine from the test solution is not larger than the peak area of fursultiamine from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and system suitability: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of fursultiamine from 10 μL of the standard solution is between 20 mm and 30 mm.

Time span of measurement: About 3 times as long as the retention time of fursultiamine.

Water Not more than 5.0 % (0.3 g, volume titration, direct titration).

Residue on Ignition Not more than 0.10 % (1 g).

Assay Weigh accurately about 55 mg each of Fursultiamine Hydrochloride and Fursultiamine Hydrochloride RS (separately determine the water in the same manner as Fursultiamine Hydrochloride), dissolve each in 50 mL of water, add exactly 10 mL each of the internal standard solution and then add water to make exactly 100 mL. Take 8 mL each of the solutions, add water to make 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fursultiamine to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of fursultiamine hydrochloride } (C_{17}H_{26}N_4O_3S_2\cdot HCl) = \text{Amount (mg) of Fursultiamine Hydrochloride RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isopropyl *p*-aminobenzoate in ethanol (95) (3 in 400)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50 °C.

Mobile phase: Dissolve 1.01 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). Take 675 mL of this solution, add 325 mL of a mixture of methanol and acetonitrile (3 : 2).

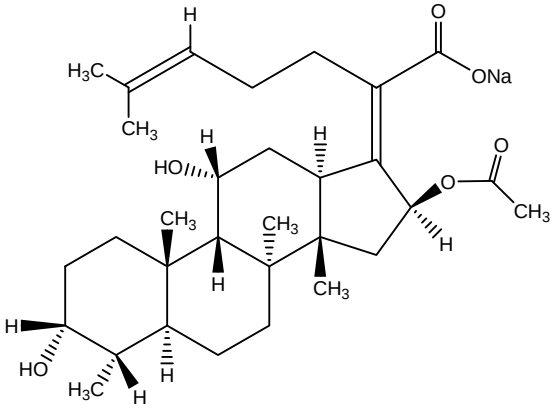
Flow rate: Adjust the flow rate so that the retention time of fursultiamine is about 9 minutes.

System suitability

Selection of the column: Proceed with 10 μL of the standard solution under the above operating conditions and calculate the resolution. Use a column giving elution of fursultiamine and the internal standard in this order with the resolution between their peaks being not less than 10.

Containers and Storage ***Containers***—Tight containers.

Fusidate Sodium



$C_{31}H_{47}NaO_6$: 538.69

Sodium(2*Z*)-2-[(3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1*H*-cyclopenta[a]phenanthren-17-ylidene]-6-methylhept-5-enoate [*751-94-0*]

Fusidate Sodium is the sodium salt of a compound having antibacterial activity produced by the growth of *Fusidium coccineum*.

Fusidate Sodium contains not less than 935 μg (potency) and not more than 969 μg (potency) per mg of fusidic acid ($C_{31}H_{48}O_6$: 516.71), calculated on the anhydrous basis.

Description Fusidate Sodium appears as white crystals or crystalline powder.

Fusidate Sodium is freely soluble in water, in methanol, or in ethanol (99.5).

Identification (1) Weigh accurately 0.10 g of Fusidate Sodium, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately 0.25 g of Diethanolamine Fusidate RS, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Per-

form the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot a suitable amount each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane, acetic acid (100), and methanol (80 : 10 : 10 : 2.5), and air-dry the plate. Spray evenly a saturated solution of antimony (III) chloride in chloroform on the plate, dry at 105 °C for 20 minutes, cool to room temperature, and examine under ultraviolet light: the spots obtained from the test solution and standard solution have the same R_f value.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(3) Fusidate Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +5 ~ +8° (a few drops of ammonia TS added to a 3 % solution, 100 mm).

pH Dissolve 125 mg (potency) of Fusidate Sodium in 10 mL of water: the pH of this solution is between 7.5 and 9.0.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Fusidate Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Proceed as directed in Purity under Fusidic Acid (total related substances not more than 2.0 %).

Water Not more than 2.0 % (1 g, volumetric titration, direct titration).

Assay Proceed as directed in the Assay under Fusidic Acid.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature between 2 and 8 °C.

Fusidate Sodium Ointment

Fusidate Sodium Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Method of Preparation Prepare as directed under Ointments, with Fusidate Sodium.

Identification (1) Proceed as directed in the Identification (1) under Fusidate Sodium. Transfer about 2 g of Fusidate Sodium Ointment to a separatory funnel, add 25 mL of petroleum ether, shake, add 5 mL of 70 % ethanol solution, shake vigorously, extract, and use the ethanol layer as the test solution.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity ***Related substances***—Proceed as directed in the Purity under Fusidic Acid (total related substances not more than 4.0 %). Weigh accurately an amount of Fusidic Acid Ointment, equivalent to about 50 mg (potency) according to the labeled potency, add 25 mL of *n*-heptane and 10 mL of the mobile phase, transfer to a separatory funnel, mix until homogeneous, filter the lower layer, and use as the test solution. Exclude any peak confirmed to be due to the excipient.

Assay Proceed as directed in the Assay under Fusidic Acid. Weigh accurately an amount of Fusidic Acid Ointment, equivalent to about 30 mg (potency) according to the labeled potency, transfer to a separatory funnel, add 10 mL of *n*-heptane, shake until homogeneous, extract with 25 mL of the mobile phase, and add the mobile phase to make exactly 50 mL. Separately, weigh accurately about 30 mg (potency) of Diethanolamine Fusidate RS, dissolve in the mobile phase to make exactly 50 mL, and use this solution s the standard solution.

Containers and Storage ***Containers***—Tight containers.

Fusidic Acid Cream

Fusidic Acid Cream contains not less than 90.0 % and not more than 120.0 % of the labeled amount of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Method of Preparation Prepare as directed under Creams, with Fusidic Acid Hydrate.

Identification (1) Proceed as directed in the Identification (2) under Fusidic Acid Hydrate. Dissolve separately 10 mg (potency) each of Fusidic Acid Cream and Diethanolamine Fusidate RS in 10 mL of water, and use these solutions as the test solution and standard solution, respectively.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH Dissolve an amount of Fusidic Acid Cream, equivalent to 1.0 g (potency) of fusidic acid, in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

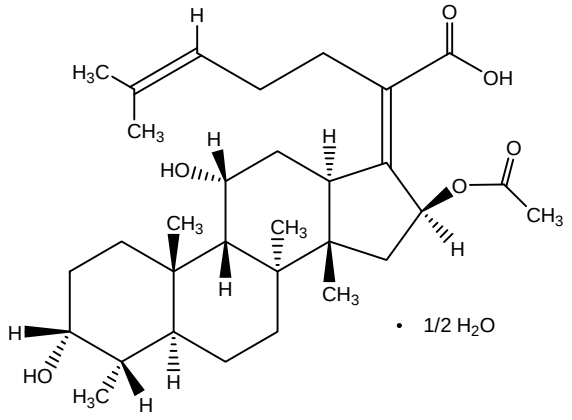
Purity ***Related substances***—Proceed as directed in the Purity under Fusidic Acid Hydrate (total related substances not more than 5.0 %). Weigh accurately an amount of Fusidic Acid Cream, equivalent to about 15 mg (potency), add 25 mL of the mobile phase, warm in

a water bath until the cream is dissolved, and shake vigorously for 15 minutes. Cool to below 10 °C, filter, discard the first 4 to 5 mL of the filtrate, and use the subsequent filtrate at room temperature as the test solution.

Assay Proceed as directed in the Assay under Fusidic Acid Hydrate. Weigh accurately an amount of Fusidic Acid Crea, equivalent to about 15 mg (potency) according to the labeled potency, add 50 mL of the mobile phase, warm in a water bath until the cream is dissolved, and shake vigorously. Cool to below 10 °C, filter, discard the first 4 to 5 mL of the filtrate, and use the subsequent filtrate at room temperature as the test solution. Separately, weigh accurately about 15 mg (potency) of Diethanolamine Fusidate RS, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Fusidic Acid Hydrate



$C_{31}H_{48}O_6 \cdot 1/2H_2O$: 525.72

(2*Z*)-2-[(3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-Acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodeca-hydro-1*H*-cyclopenta[a]phenanthren-17-ylidene]-6-methylhept-5-enoic acid [*6990-06-3*]

Fusidic Acid Hydrate contains not less than 975 μg (potency) per mg of fusidic acid ($C_{31}H_{48}O_6$: 516.71), calculated on the anhydrous basis.

Description Fusidic Acid Hydrate appears as white crystalline powder.
Fusidic Acid Hydrate is freely soluble in ethanol (95) or in chloroform, sparingly soluble in ether, and practically insoluble in water.

Identification (1) Dissolve separately 50 mg (potency) each of Fusidic Acid Hydrate and Diethanolamine Fusidate RS in 1 mL of chloroform, and perform the test as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 20 mg (potency) of Fusidic Acid Hydrate in 10 mL of ethanol (99.5), and use this solution as the test solution. Separately, dissolve 20 mg (potency) of Diethanolamine Fusidate RS in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot a suitable amount each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane, acetic acid (100), and methanol (80 : 10 : 10 : 2.5), and air-dry the plate. Spray 10 % sulfuric acid-ethanol solution on the plate, dry at 110 °C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the spots obtained from the test solution and standard solution have the same R_f value.

(3) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity ***Related substances***—Proceed as directed in the Assay. Determine the area of each peak from the test solution by the automatic integration method, and calculate the amount of related substances: the total amount of related substances is not more than 2.0 %. Weigh accurately about 50 mg (potency) of Fusidic Acid Hydrate, dissolve in the mobile phase to make 10 mL, and use this solution as the test solution.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance with a relative retention time of 0.3 to 0.35, other than the principal peak

A_S: Total area of all peaks

Exclude any peak with an area not more than 0.01 % of the area of fusidic acid.

Detection sensitivity: To 1 mL of the standard solution obtained in the Assay add the mobile phase to make 100 mL. Adjust the detection sensitivity so that the peak height of fusidic acid obtained from 10 μL of this solution under the above operating conditions is about 5 mm.

Water 1.4 ~ 2.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1.0 g).

Assay Weigh accurately about 50 mg (potency) each of Fusidic Acid Hydrate and Diethanolamine Fusidate

RS, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of fusidic acid in each solution.

$$\text{Amount [μg (potency)] of fusidic acid } (C_{31}H_{48}O_6) = \text{Amount [μg (potency)] of Fusidic Acid RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile, 0.05 mol/L phosphoric acid solution, and methanol (5 : 4 : 1)

Flow rate: 1.2 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature between 2 and 8 °C.

Fusidic Acid Ophthalmic Solution

Fusidic Acid Ophthalmic Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Method of Preparation Prepare as directed under Ophthalmic Solutions, with Fusidic Acid Hydrate.

Identification The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

pH 5.0 ~ 6.5.

Sterility Test It meets the requirement.

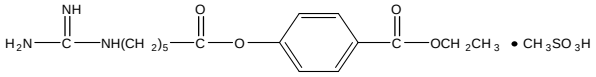
Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Ophthalmic Solutions It meets the requirement.

Assay Proceed as directed in the Assay under Fusidic Acid Hydrate. Weigh accurately an amount of Fusidic Acid Ophthalmic Solution, equivalent to about 10 mg (potency) according to the labeled potency, add 40 mL of the mobile phase, shake, add 0.5 g of potassium nitrate, add the mobile phase to make exactly 50 mL, shake for 1 minute, filter through a glass filter, and use the filtrate as the test solution. Separately, weigh accurately about 10 mg (potency) of Diethanolamine Fusidate RS, proceed in the same manner as for the test solution, and use this solution as the standard solution.

Containers and Storage ***Containers***—Tight containers.

Gabexate Mesilate



$C_{16}H_{23}N_3O_4 \cdot CH_4O_3S$: 417.48

Ethyl 4-[6-(diaminomethylideneamino)hexanoyl - oxy]benzoate;methanesulfonic acid [*56974-61-9*]

Gabexate Mesilate, when dried, contains not less than 98.5 % and not more than 101.0 % of gabexate mesilate ($C_{16}H_{23}N_3O_4 \cdot CH_4O_3S$).

Description Gabexate Mesilate appears as white crystals or crystalline powder.

Gabexate Mesilate is very soluble in water, freely soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Take 4 mL of a solution of Gabexate Mesilate (1 in 2000), add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS and allow to stand for 10 minutes: a red color is observed.

(2) Dissolve 1 g of Gabexate Mesilate in 5 mL of water, add 2 mL of sodium hydroxide TS and heat on a water-bath for 5 minutes. After cooling, add 2 mL of dilute nitric acid and 5 mL of ethanol (95), shake, add 5 drops of iron (III) chloride TS and shake: a purple color is observed.

(3) Determine the absorption spectra of solutions of Gabexate Mesilate and Gabexate Mesilate RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Gabexate Mesilate responds to the Qualitative Tests (1) for mesilate.

Melting Point 90 ~ 93 ºC

pH Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the pH of this solution is between 4.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Gabexate Mesilate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard

lead solution (not more than 10 ppm).

(3) ***Arsenic***—Dissolve 2.0 g of Gabexate Mesilate in 20 mL of 1 mol/L hydrochloric acid by heating on a water-bath and continue the heating for 20 minutes. After cooling, centrifuge and use 10 mL of the clear supernatant liquid as the test solution. Perform the test (not more than 2 ppm).

(4) ***Ethyl parahydroxybenzoate***—Weigh 50 mg of Gabexate Mesilate, previously dried and dissolve in dilute ethanol to make exactly 100 mL. Pipet 5.0 mL of this solution, add exactly 5 mL of the internal standard solution and use this solution as the test solution. Separately, dissolve 5.0 mg of ethyl parahydroxybenzoate in dilute ethanol to make exactly 100 mL. Pipet 1.0 mL of this solution and add dilute ethanol to make exactly 20 mL. Take exactly 5 mL of this solution, add exactly 5 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 3 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the operating conditions in the Assay and calculate the ratios, Q_T and Q_S, of the peak area of ethyl parahydroxybenzoate to that of the internal standard, respectively: Q_T is not larger than Q_S.

Internal standard solution—A solution of butyl parahydroxybenzoate in diluted ethanol (1 in 5000).

(5) ***Related substances***—Dissolve 0.20 g of Gabexate Mesilate in 5 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate until it has no acetic odor. Spray evenly a solution of 8-quinolinol in acetone (1 in 1000) on the plate and after air-drying, spray evenly bromine-sodium hydroxide TS: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg of Gabexate Mesilate and Gabexate Mesilate RS, previously dried and dissolve each in dilute ethanol to make exactly 100 mL. Pipet 5.0 mL each of these solutions, add exactly 5 mL each of the internal standard solution and use these solutions as the test solution and the standard solution, respectively. Perform the test with 3 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of gabexate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of gabexate mesilate } (C_{16}H_{23}N_3O_4.CH_4O_3S) = \text{Amount (mg) of Gabexate Mesilate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in diluted ethanol (1 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 245 nm)

Column: A stainless steel column, about 5 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol, a solution of sodium lauryl sulfate (1 in 1000), a solution of sodium 1-heptane sulfonate (1 in 200) and acetic acid (100) (540 : 200 : 20 : 1).

Flow rate: Adjust the flow rate so that the retention time of Gabexate is about 13 minutes.

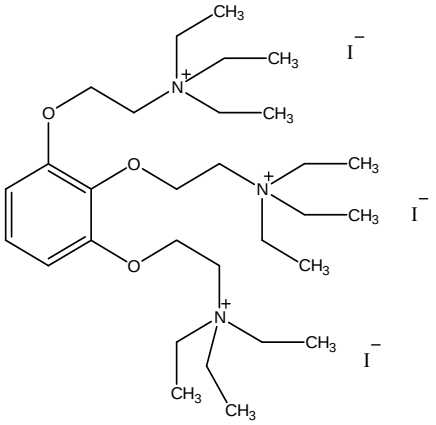
System suitability

System performance: When the procedure is run with 3 μL of the standard solution, as directed under the above operating condition, the internal standard and gabexate are eluted in this order with the resolution between their peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 3 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of gabexate to that of the internal standard is not more than 1.0 %

Containers and Storage ***Containers***—Tight containers.

Gallamine Triethiodide



$C_{30}H_{60}I_3N_3O_3$: 891.53

2-[2,3-*bis*[2-(Triethylazanimyl)ethoxy]phenoxy] ethyltriethylazanium triiodide [*65-29-2*]

Gallamine Triethiodide contains not less than 98.0 % and not more than 101.0 % of gallamine triethiodide ($C_{30}H_{60}I_3N_3O_3$), calculated on the dried basis.

Description Gallamine Triethiodide is a white, amorphous power and is odorless.
Gallamine Triethiodide is very soluble in water, sparingly soluble in ethanol (95) and very slightly soluble in chloroform.
Gallamine Triethiodide is hygroscopic.

Identification (1) Determine the infrared spectra of Gallamine Triethiodide and Gallamine Triethiodide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Gallamine Triethiodide (1 in 100) responds to the Qualitative Tests for iodide.

pH Dissolve 1 g of Gallamine Triethiodide in 50 mL of water: the pH of this solution is between 5.3 and 7.0.

Purity (1) ***Clarity and color of solution***—A solution of Gallamine Triethiodide (1 in 100) is colorless and clear.

(2) ***Heavy metals***—Proceed with 1.0 g of Gallamine Triethiodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 1.5 % (1 g, 100 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 25 mg each of Gallamine Triethiodide and Gallamine Triethiodide RS, dissolve in the mobile phase to make exactly 25 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peak of gallamine triethiodide in the test solution, A_T, and in the standard solution, A_S, respectively.

$$\text{Amount (mg) of gallamine triethiodide } (C_{30}H_{60}I_3N_3O_3) = 25 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of gallamine triethiodide in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3-10 μm in particle diameter).

Mobile phase: A mixture of sodium perchlorate buffer and acetonitrile (69:31).

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates is not less than 5000 with the symmetry factor being not more than 1.4.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of the peak of gallamine triethiodide is not more than 2.0 %.

Sodium perchlorate buffer—Dissolve sodium perchlorate in water to render the concentration of 0.14 mol/L and adjust the pH to 3.0 by addition of 10 mol/L sodium hydroxide TS or of 0.05 mol phosphoric acid.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

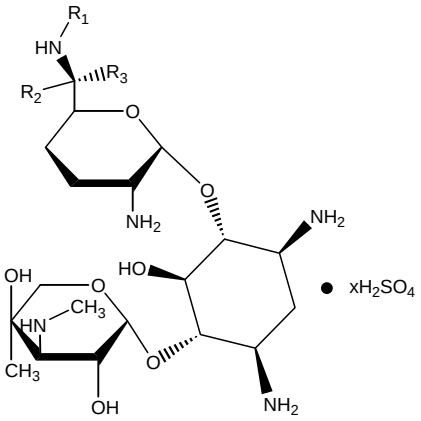
Gallium Citrate (^{67}Ga) Injection

Gallium (^{67}Ga) Citrate Injection is an aqueous solution for injection containing Gallium (^{67}Ga) Citrate Injection.
It conforms to the requirements of Gallium (^{67}Ga) Citrate Injection specified in the Korean Pharmaceutical Codex.
The Insoluble Particulate Matter Test for Injections is not applied to this injection.

Description Gallium (^{67}Ga) Citrate Injection is a clear, colorless or pale red liquid

Bacterial Endotoxins Less than 175/V EU/mL of Gallium (^{67}Ga) Citrate Injection, where V is the maximum recommended dose per mL during the effective time.

Gentamicin Sulfate



Gentamicin C_1:	$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = H$
Gentamicin C_{1a}:	$R_1 = H$	$R_2 = H$	$R_3 = H$
Gentamicin C_2:	$R_1 = H$	$R_2 = CH_3$	$R_3 = H$
Gentamicin C_{2a}:	$R_1 = H$	$R_2 = H$	$R_3 = CH_3$
Gentamicin C_{2b}:	$R_1 = CH_3$	$R_2 = H$	$R_3 = H$

(3*R*,4*R*,5*R*)-2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-{[(2*R*,3*R*,6*S*)-3-amino-6-[(1R)-1-(methylamino)ethyl]oxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}-5-methyl-4-(methylamino)oxane-3,5-diol; sulfuric acid [*1405-41-0*]

Gentamicin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Micromonospora purpurea* or *Micromonospora echinospora*.
Gentamicin Sulfate contains not less than 590 μg (potency) per mg of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60), calculated on the dried basis

Description Gentamicin Sulfate is a white to pale yellow powder.
Gentamicin Sulfate is very soluble in water, and practically insoluble in ethanol (99.5).
Gentamicin Sulfate is hygroscopic.

Identification (1) Dissolve 50 mg of Gentamicin Sulfate in 1 mL of water, and add 2 drops of the solution of 1-naphthol in ethanol (95) (1 in 500). Gently superimpose the solution on 1 mL of sulfuric acid: a purple-blue color develops at the zone of contact.

(2) Dissolve 50 mg each of Gentamicin Sulfate and Gentamicin Sulfate RS in 10 mL of water, and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Place a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) into a separatory funnel, shake and allow to stand at room temperature for not less than 1 hour. Take 20 mL from the lower layer of this solution, add 0.5 mL of methanol and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm with the developing chamber cover open about 20 mm^2 and without placing filter paper inside the container, and air-dry the plate. Expose the plate to iodine vapor, cover the plate with a glass plate and compare the spots: the 3 spots obtained from the test solution show the same R_f value and color as each of the spots obtained from the standard solution.

(3) Dissolve 50 mg of Gentamicin Sulfate in 5 mL of water and add 5 mL of barium chloride TS: a white precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +107 ~ +121° (0.25 g calculated on the dried basis, water, 25 mL, 200 mm)

pH The pH of a solution obtained by dissolving 0.20 g of Gentamicin Sulfate in 5 mL of water is between 3.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Gentamicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Gentamicin Sulfate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50 mg of Gentamicin Sulfate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Separately, place a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separatory funnel, shake and allow to stand at room temperature for not less than 1 hour. Take 20 mL from the lower layer of this solution, add 0.5 mL of methanol and use this as the developing solvent. Develop the plate to a distance of about 17 cm with the developing chamber cover open about 20 mm^2 and without placing filter paper inside the container, and air-dry the plate. Expose the plate to iodine vapor, cover the plate with a glass plate and compare the spots: the spots other than the spots of gentamicin C_1 (R_f value of about 0.3), gentamicin C_2 (R_f value of about 0.2) and gentamicin C_{1a} (R_f value of about 0.1) obtained from the test solution are not more intense than the spot of gentamicin C_2 obtained from the standard solution.

Sterility Test It meets the requirement, when Gentamicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of gentamicin, when Gentamicin Sulfate is used in a sterile preparation.

Loss on Drying Not more than 18.0 % (0.15 g, reduced pressure not exceeding 0.67 kPa, 110 °C, 3 hours), avoid moisture absorption when taking the sample.

Content Ratio of Gentamicins Weigh accurately an amount of Gentamicin Sulfate and Gentamicin Sulfate RS, dissolve in water and make a solution so that each mL contains 0.65 mg (potency). To 10 mL of each of these solutions add 5 mL of 2-propanol and 4 mL of the O-Phthalaldehyde solution, mix, and add 2-propanol to obtain 25 mL of solutions. Heat at 60 °C in water bath for 15 minutes, and cool. The resulting solutions are used as the test solution and the standard solution. Perform the test with these solutions as directed under Liquid Chromatography according to the following operating conditions and obtain the peak areas, A_f, of gentamicins (gentamicin C_1: 25 ~ 50 %, gentamicin C_{1a}: 10 ~ 35 %, gentamicin C_2 and C_{2a}: 25 ~ 55 %).

$$\text{Content (\%) of gentamicin } C_1,\ C_{1a},\ C_2 \text{ and } C_{2a} = \frac{A_f}{A_S} \times 100$$

A_f : the peak area response corresponding to the particular gentamicin

A_S : the sum of the area responses of all four peaks

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 330 nm)

Column: A stainless steel column, about 5 mm in internal diameter and about 100 mm in length, packed with octadecylsilanized silica gel or fine ceramic particle for liquid chromatography(5 ~ 10 μm in particle diameter)

Mobile phase: Mix 700 mL of methanol, 250 mL of water and 50 mL of glacial acetic acid. Dissolve 5 g of sodium 1-heptanesulfonate in this solution.

Flow rate: about 1.5 mL per minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the capacity factor determined from the gentamicin C_1 peak is between 2 and 7, the column efficiency determined from the gentamicin C_2 peak is mot less than 1200 theoretical plates, and the resolution, R, between any two peaks is not less than 1.25. The elution order is gentanicin C_1, gentanicin C_{1a}, gentamicin C_{2a}, and gentamicin C_2.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the operating conditions, the relative standard deviation of any peak area is not more than 2.0 %.

o-Phthalaldehyde solution—Dissolve 1.0 g of *o*-phthalaldehyde in 5 mL of methanol and add 95 mL of 0.4 mol/L boric acid solution, previously adjusted to pH 10.4 with 8 mol/L potassium hydroxide. Add 2 mL of thioglycolic acid then add 8mol/L potassium hydroxide to adjust to pH 10.4.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer-

Peptone	6.0 g	Glucose	2.0 g
Yeast extract	3.0 g	Sodium chloride	10.0 g
Meat extract	1.5 g	Agar	15.0 g
Water	1000 mL		

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(2) Agar medium for transferring test organisms- Use the medium in I 2 1) (2) ② under Microbial Assay for Antibiotics.

(3) Test organism- *Staphylococcus epidermidis* ATCC 12228

(4) Weigh accurately an amount of Gentamicin Sulfate, equivalent to about 25 mg (potency) and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution containing 1 mg gentamicin per 1 mL (potency) and use the solution as the test stock solution. Take exactly a suitable amount of the test stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately an amount of Gentamicin Sulfate RS, equivalent to about 25 mg (potency) and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution containing 1 mg gentamicin per 1 mL (potency) and use the solution as the standard stock solution. Keep the standard stock solution at not exceeding 15 °C and use within 30 days. Take exactly a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Gentamicin Sulfate Cream

Gentamicin Sulfate Cream contains not less than 90.0 % and not more than 120.0 % of the labeled amount of gentamicin.

Method of Preparation Prepare as directed under Creams, with Gentamicin Sulfate.

Identification Weigh a suitable amount of Gentamicin Sulfate Cream, put into a blender, add a suitable amount of 0.1 mol/L phosphate buffer solution (pH 8.0), previously warmed to between 70 °C and 85 °C, and blend on high speed for 3 to 5 minutes. Take this solution and proceed as directed in the Identification (2) under Gentamicin Sulfate.

Assay Proceed as directed in the Assay under Gentamicin Sulfate.

Containers and Storage ***Containers***—Tight containers.

Gentamicin Sulfate Injection

Gentamicin Sulfate Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of gentamicin.

Method of Preparation Prepare as directed under Injections, with Gentamicin Sulfate.

Description Gentamicin Sulfate Injection is a clear and colorless to pale yellow liquid.

Identification Weigh an amount of Gentamicin Sulfate Injection equivalent to 0.1 g (potency) of Gentamicin Sulfate according to the labeled amount, and 0.1 g (potency) of Gentamicin Sulfate RS, and perform the test as directed in Identification (2) under Gentamicin Sulfate.

pH 3.0 ~ 5.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of gentamicin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Gentamicin Sulfate. Pipet a suitable amount of Gentamicin Sulfate Injection according to the labeled potency and add 0.1 mol/L phosphate buffer solution (pH 8.0) to obtain a solution of suitable concentration. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer solution (pH 8.0) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Gentamicin Sulfate Ophthalmic Ointment

Gentamicin Sulfate Ophthalmic Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of gentamicin.

Method of Preparation Prepare as directed under Ophthalmic Ointments, with Gentamicin Sulfate.

Identification Weigh a suitable amount of Gentamicin Sulfate Ophthalmic Ointment, add a suitable amount of 0.1 mol/L phosphate buffer solution (pH 8.0), previously warmed to between 70 °C and 85 °C, disperse well and centrifuge. Take the clear supernatant liquid and proceed with as directed in the Identification (2) under Gentamicin Sulfate.

Sterility Test It meets the requirement.

Test for Metal Particles It meets the requirement.

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Gentamicin Sulfate. Transfer an accurately weighed portion of Gentamicin Sulfate Ophthalmic Ointment, equivalent to about 1.0 mg (potency) according to the labeled potency, to a separatory funnel. Add 50 mL of ether and shake until uniform. Extract with three 25 mL volumes of 0.1 mol/L phosphate buffer solution (pH 8.0), combine the extracts and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make 100 mL. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer solution (pH 8.0) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Gentamicin Sulfate Ophthalmic Solution

Gentamicin Sulfate Ophthalmic Solution is an aqueous ophthalmic solution. Gentamicin Sulfate Ophthalmic Solution contains not less than 90.0 % and not more than 110.0 % of the labeled amount of gentamicin.

Method of Preparation Prepare as directed under Ophthalmic Solutions, with Gentamicin Sulfate.

Identification To a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to 10 mg (potency) of Gentamicin Sulfate according to the labeled amount, add water to make 5 mL, and use this solution as the test solution. Separately, dissolve an amount of Gentamicin Sulfate RS, equivalent to 10 mg (potency), in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100 °C for 5 minutes: the three principal spots obtained from the test solution are the same with the corresponding from the standard solution in color tone and the R_f value, respectively.

pH 5.5 ~ 7.5.

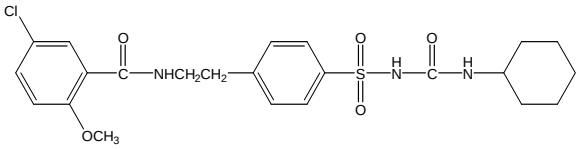
Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Assay Proceed as directed in the Assay under Gentamicin Sulfate. Pipet a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to about 12 mg (potency) of Gentamicin Sulfate and add 0.1 mol/L phosphate buffer solution (pH 8.0), to make a solution so that each mL contains about 1mg (potency). Pipet a suitable volume of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0), to make solutions so that each mL contains 4.0 μg (potency) and 1.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively.

Containers and Storage ***Containers***—Tight containers.

Glibenclamide



$C_{23}H_{28}ClN_3O_5S$: 494.00

5-Chloro-*N*-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-2-methoxybenzamide [*10238-21-8*]

Glibenclamide, when dried, contains not less than 98.5 % and not more than 101.0 % of glibenclamide ($C_{23}H_{28}ClN_3O_5S$).

Description Glibenclamide is a white to pale yellow crystalline powder.
Glibenclamide is freely soluble in *N,N*-dimethylformamide, sparingly soluble in chloroform, slightly soluble in methanol or in ethanol (95) and practically insoluble in water or in ether.

Identification (1) Determine the absorption spectra of solutions of Glinbenclamide and Glibenclamide RS in methanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Glibenclamide and Glibenclamide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Glibenclamide as directed under the Flame Coloration Test (2): a green color is observed.

Melting Point 168 ~ 173 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Glibenclamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.20 g of Glibenclamide in 20 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for Thin-layer chromatoghraphy. Develop the plate with a mixture of 1-propanol, chloroform and diluted ammonia TS (4 in 5) (11 : 7 : 2) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the

spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

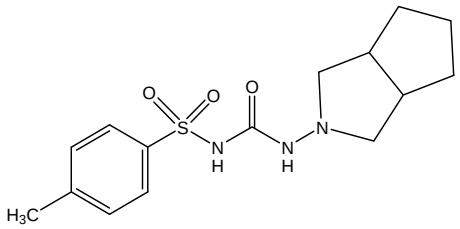
Residue on Ignition Not more than 0.5 % (1 g)

Assay Weigh accurately about 0.9 g of Glibenclamide, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a black determination with a solution prepared by adding 18 mL of water to 50 mL of *N,N*-dimethyl-formamide and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 49.40 mg of $C_{23}H_{28}ClN_3O_5S$

Containers and Storage ***Containers***—Tight containers.

Gliclazide



$C_{15}H_{21}N_3O_3S$: 323.41

N-((Hexahydrocyclopenta[c]pyrrol-2(1*H*)-yl)carbamoyl)-4-methylbenzenesulfonamide
[*21187-98-4*]

Gliclazide, when dried, contains not less than 99.0 % and not more than 101.0 % of gliclazide ($C_{15}H_{21}N_3O_3S$).

Description Gliclazide is a white crystalline powder. Gliclazide is freely soluble in dichloromethane, sparingly soluble in acetone, slightly soluble in ethanol (95) and practically insoluble in water.

Identification Determine the infrared spectra of Gliclazide and Gliclazide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 165 ~ 169 °C

Purity **(1)** ***Heavy metals***—Proceed with 1.5 g of Gliclazide according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substance I***—Weigh accurately 0.4 g of Gliclazide, dissolve in 2.5 mL of dimethylsulfoxide, add water to make exactly 10 mL, mix by shaking for 10 minutes, allow the solution to stand at 4 °C for 30 minutes, filter and use the filtrate as the test solution. Weigh 20.0 mg of gliclazide related substance I RS [2-nitroso-octahydrocyclopenta[*c*]pyrrole] and add dimethylsulfoxide to make exactly 100 mL. To 1.0 mL of this solution, add 12 mL of dimethylsulfoxide and water to make exactly 50 mL, and use this solution as the standard solution (1). To 1.0 mL of the standard solution (1), add 12 mL of dimethylsulfoxide and water to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with 50 μL each of the test solution and the standard solution (2) as directed under Liquid Chromatography according to the operating conditions directed in the Related substances under the Purity: the area of the related substance I peak obtained from the test solution is not greater than that from the standard solution (2) (2 ppm).

(3) ***Related substances***—Weigh accurately 50 mg of Gliclazide, dissolve in 23 mL of acetonitrile, add water to make exactly 50 mL, and use this solution as the test solution. To 1.0 mL of the test solution and add a mixture of water and acetonitrile (55 : 45) to make exactly 100 mL. Pipet 10.0 mL of the solution, add a mixture of water and acetonitrile (55 : 45) to make exactly 100 mL, and use this solution as the standard solution (1). Dissolve 5 mg of Gliclazide and 15 mg of gliclazide related substance II RS [1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(2-methylphenyl)sulfonyl]urea] in 23 mL of acetonitrile, add water to make 50 mL, pipet 1.0 mL of this solution, add a mixture of water and acetonitrile (55 : 45) to make exactly 20 mL and use this solution as the standard solution (2). Dissolve 10.0 mg of gliclazide related substance II RS in 45 mL of acetonitrile and add water to make exactly 100 mL, pipet 1.0 mL of this solution, add a mixture of water and acetonitrile (55 : 45) to make exactly 100 mL and use this solution as the standard solution (3). Perform the test with 20 μL each of the test solution, the standard solutions (1) and (3) as directed under Liquid Chromatography according to the following operating conditions and determine the area of peaks from the these solutions: the peak area corresponding to the related substance II obtained from the test solution is not larger than the area of the principal peak obtained from the standard solution (3) (0.1 %), the area of any peak other than the principal peak or the related substance II peak from the test solution is not larger than the area of the principal peak from the standard solution (1) (0.1 %), and total area of these other peaks from the test solution is not larger than twice the area of the principal peak from the standard solution (1) (0.2 %). Disregard any peak having the area not more than 0.2 times the area of the principal peak from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile, trifluoroacetic acid and triethylamine (55:45:0.1:0.1).

Flow rate: 0.9 mL/minute.

System suitability

System performance: Proceed with 20 μL of the standard solution (2) under the above operating conditions and adjust the sensitivity of the system so that the heights of the two principal peaks obtained from the standard solution (2) are not less than 50 % of the full scale of the recorder. Under this operating condition, the resolution between the two principal peaks is not less than 1.8.

Loss on Drying Not more than 0.25 % (1 g, 105 ºC, 2 hours).

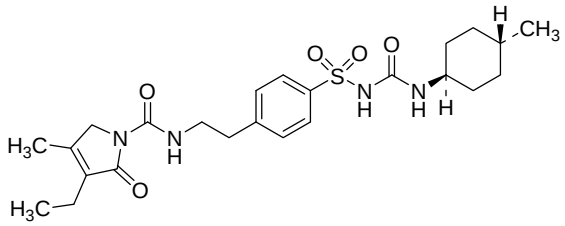
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Gliclazide, dissolve in 50 mL of glacial acetic acid and titrate with 0.1 mol/L perchlroic acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of perchloric acid VS
= 32.34 mg of $C_{15}H_{21}N_3O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Glimepiride



$C_{24}H_{34}N_4O_5S$: 490.62

3-Ethyl-4-methyl-*N*-(4-(*N*-(((1r,4r)-4-methylcyclohexyl)carbamoyl)sulfamoyl)phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide
[*93479-97-1*]

Glimepiride contains not less than 98.0 % and not more than 102.0 % of Glimepiride ($C_{24}H_{34}N_4O_5S$), calculated on the anhydrous basis.

Description Glimepiride appears as a white crystalline powder.

Glimepiride is slightly soluble in dichloromethane, very slightly soluble in methanol and in ethanol (99.5) and practically insoluble in water.

Melting point—About 202 °C (with decomposition)

Identification (1) Determine the absorption spectra of the solution of Glimeperide and Glimeperide RS in methanol (1 in 125000) as directed under Ultraviolet-visible Spectrophotometry and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Glimeperide and Glimeperide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Glimepiride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Glimepiride isomer***—Dissolve 10 mg of Glimepiride in 5 mL of dichloromethane, add the mobile phase to make 20.0 mL and use this solution as the test solution. Pipet 1.0 mL of test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 to Glimeperide, obtained from the test solution is not larger than 3/4 times the peak area of Glimepiride from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 3 mm in internal diameter and 15 cm in length, packed with dihydropropane silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of heptanes, 2-propanol and acetic acid (100) (900:100:1)

Flow rate: Adjust the flow rate so that the retention time of Glimepiride is about 14 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution and add the mobile phase to make exactly 10 mL. Confirm that the peak area of Glimepiride obtained with 10 μL of this solution is equivalent to 33 % to 65 % of that with 10 μL of the standard solution.

System performance: When the procedure is run

with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of Glimepiride are not less tham 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 2.0 %.

(3) ***Related substances***—Keep the test solution and the standard solution below 4 °C after preparing. Dissolve 20 mg of Glimepiride in 100 mL of a mixture of acetonitrile and water (4:1) and use this solution as the test solution. Pipet 1 mL of this solution and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the peak area of the peak, having the related retention time of about 0.25 to Glimepiride, obtained from the test solution is not larger than 4 times the peak area of Glimepiride from the standard solution, the area of peak, having the relative retention time of about 1.1 is not larger than 2 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 0.32, is not larger than 1.5 times the peak area of glimepiride from the standard solution, the area of peak other than glimepiride and above mentioned peak from the test solution is not larger than the peak of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak, having the relative retention time of about 0.25 to Glimepiride, from the test solution is not larger than 5 times the peak area of glimepiride from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution and add the mobile phase to make exactly 10 mL. Confirm that the peak area of Glimepiride obtained with 20 μL of this solution is equivalent to 35 to 65 % of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 2.0 %.

Water Not more than 0.5 % (0.25 g, coulometric titration)

Residue on ignition Not more than 0.2 % (1 g)

Assay Weigh accurately about 20 mg of each of Glimepiride and Glimepiride RS (previously determine the water content), dissolve each substance in a mixture of acetonitrile and water (4:1) to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL of each of test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of Glimepiride from each solution.

$$\text{Amount (mg) of Glimepiride } (C_{24}H_{34}N_4O_5S) = \text{Amount (mg) of Glimepiride RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.5 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, adjust the pH to 2.5 with phosphoric acid and add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of Glimepiride is about 17 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Glimepiride Tablets

Glimepiride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of Glimepiride ($C_{24}H_{34}N_4O_5S$: 490.62).

Method of Preparation Prepare as directed under Tablets, with Glimepiride.

Identification To a quantity of powdered Glimepiride Tablets, equivalent to 20 mg of Glimepiride according to the labeled amount, add 40 mL of acetonitrile, shake for 15 minutes and centrifuge. Evaporate the clear supernatant liquid on a water-bath under reduced pressure. Add 1 mL of water to the residue, suspend and filter under reduced pressure. Wash the residue with 1 mL of water, dry at 105 °C for 1 hour and determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at wavenumbers of about 3370 cm^{-1}, 3290 cm^{-1}, 2930 cm^{-1}, 1708 cm^{-1}, 1674 cm^{-1}, 1347 cm^{-1}, 1156 cm^{-1} and 618 cm^{-1}.

Purity ***Related substances***—Keep the test solution and the standard solution below 4 °C after preparation. To a quantity of powdered Glimepiride Tablets, equivalent to 9 mg of Glimepiride according to the labeled amount, wet with 0.5 mL of water, add a mixture of acetonitrile and water (4:1) to make 50 mL, shake, centrifuge, and use the supernatant liquid as the test solution. Pipet 1 mL of the test solution and standard aolution, add a mixture of acetonitrile and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 mL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the related retention time of about 0.3 relative to Glimepiride, obtained from the test solution is not larger than 2.6 times the peak area of Glimepiride from the standard solution, the area of the peak other than Glimepiride and the peak mentioned above from the test solution is not larger than 3/10 times the peak area of Glimepiride and the peak mentioned above from the test solution is not larger than the peak area of Glimepiride from the standard solution, and the total area of the peaks other than Glimepiride from the test solution is not larger than 3 times the peak area of Glimepiride from the standard solution.

Operating conditions

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust the flow rate so that the retention time of Glimepiride is about 12 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution and add the mobile phase to make exactly 20 mL. Confirm that the peak area of Glimepiride obtained with 5 μL of this solution is equivalent to 7 % to 13 % of that with the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of Glimepiride

Dissolution Test Perform the test with 1 tablet of Glimepiride Tablets at 50 revolutions per minute according to method 2, using 900 mL of pH 7.5 disodium hydrogen phosphate-citric acid buffer as the dissolution solution. Take not less than 20 mL of the dissolved solution 15 minutes after the start of the test and filter through a membrane filter with a pore size of not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate and add the dissolution solution to make exactly V' mL of a solution containing about 1.1 μg of Glimepiride ($C_{24}H_{34}N_4O_5S$) per mL according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Glimepiride RS (previously determine the water content), and dissolve in acetonitrile to make exactly 100 mL. Pipet 2 mL of this solution, add 8 mL of acetonitrile, and add the dissolution solution to make exactly 200 mL. Pipet 10 mL of this solution, add the dissolution solution to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of Glimepiride of both solutions. The dissolution rate of Glimepiride Tablets in 15 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of Glimepiride ($C_{24}H_{34}N_4O_5S$)
= Amount (mg) of Glimepiride RS,
calculated on the anhydrous basis $\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{9}{2}$

C: Labeled amount (mg) of Glimepiride ($C_{24}H_{34}N_4O_5S$) in 1 tablet

Operating conditions

Detector, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above

operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 1.5 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

To one tablet of Glimepiride Tablets add V / 20 mL of water, disintegrate, add V / 2 mL of a mixture of acetonitrile and water (4:1) and shake. To this solution, add exactly V / 10 mL of the internal standard solution, add a mixture of acetonitrile and water (4:1) to make V mL so that each mL contains about 50 μg of Glimepiride ($C_{24}H_{34}N_4O_5S$), centrifuge, and use the supernatant liquid as the test solution. Separately, weigh accurately about 20 mg of Glimepiride RS (previously determine the water content) and dissolve in a mixture of acetonitrile and water (4:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of acetonitrile and water (4:1) to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of Glimepiride ($C_{24}H_{34}N_4O_5S$)
= Amount (mg) Glimepiride RS,
calculated on the anhydrous basis $\times \frac{Q_T}{Q_S} \times \frac{V}{400}$

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (4:1) (1 in 1000)

Assay Weigh accurately the mass of not less than 20 Glimepiride Tablets, and powder. Weigh accurately a potion of the powder, equivalent to about 3 mg of glimepiride ($C_{24}H_{34}N_4O_5S$), add 3 mL of water, and shake with 30 mL of a mixture of and water (4:1). Add exactly 6 mL of the internal standard solution, and add a mixture of acetonitrile and water (4:1) to make 50 mL, centrifuge, and use the supernatant liquid as the test solution. Separately, weigh accurately about 20 mg of Glimepiride RS, (separately, determine the water in the same manner as Glimepiride), dissolve in a mixture of acetonitrile and water (4:1) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 6 mL of the intenal standard solution, add a mixture of and water (4:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 mL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of glimepiride to that of the internal standard.

Amount (mg) of Glimepiride ($C_{24}H_{34}N_4O_5S$)
= Amount (mg) of Glimepiride RS
on the anhydrous basis $\times \frac{Q_T}{Q_S} \times \frac{3}{20}$

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (4:1) (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4 mm in internal diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.5 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, add 500 mL of acetonitrile and adjust the pH to 3.5 with diluted phosphoric acid (1 in 5).

Flow rate: Adjust the flow rate so that the retention time of Glimepiride is about 10 minutes.

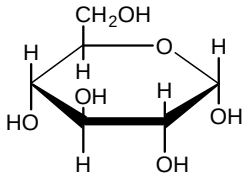
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and Glimepiride are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Glimepiride is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Glucose



D-Glucopyranose $C_6H_{12}O_6$: 180.16

(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanal [*50-99-7*]

Glucose is α-D-glucopyranose, β-D-glucopyranose or a mixture of them.

Glucose, when dried, contains not less than 99.5 % and not more than 101.0 % of glucose [D-glucopyranose ($C_6H_{12}O_6$)].

Description Glucose appears as white crystals or crystalline powder, is odorless and has a sweet taste.

Glucose is freely soluble in water, soluble in hot etha-

nol, slightly soluble in ethanol (95) and practically insoluble in ether.

Identification Add 2 to 3 drops of a solution of Glucose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

Purity (1) ***Clarity and color of solution***—Add 25.0 g of Glucose to 30 mL of water in a Nessler tube, warm at 60 ºC in a water-bath until solution is obtained, cool and add water to make 50 mL: the solution is clear and has no more color than the following control solution.

Control solution—Take a mixture of 1.0 mL of cobalt (II) chloride hexahydrate stock CS, 3.0 mL of iron (III) chloride hexahydrate stock CS and 2.0 mL of cupric sulfate stock CS, add water to make 10.0 mL. Pipet 3.0 mL of this solution and add water to make 50 mL.

(2) ***Acid***—Dissolve 5.0 g of Glucose in 50 mL of freshly boiled and cooled water and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color is observed.

(3) ***Chloride***—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(4) ***Sulfate***—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(5) ***Heavy metals***—Proceed with 5.0 g of Glucose according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 4 ppm).

(6) ***Arsenic***—Dissolve 1.5 g of Glucose in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat in a water-bath for 5 minutes and concentrate to 5 mL. After cooling, perform the test with this solution as the test solution (not more than 1.3 ppm).

(7) ***Dextrin***—Take 1.0 g of Glucose, add 20 mL of ethanol (95) and boil under a reflux condenser: the solution is clear.

(8) ***Soluble starch and sulfite***—Dissolve 1.0 g of Glucose in 10 mL of water and add 1 drop of iodine TS: a yellow color is observed.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 6 hours).

Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 10 g of Glucose, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, allow to stand for 30 minutes and determine the optical rotation, α_D, of this solution at 20 ± 1 ºC in a 100-mm cell as directed under the Optical Rotation Determination.

$$\text{Amount (mg) of } C_6H_{12}O_6 = \alpha_D \times 1895.4$$

Containers and Storage ***Containers***—Tight containers.

Glucose Injection

Glucose Injection is an aqueous solution for injection. Glucose Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of glucose ($C_6H_{12}O_6$: 180.16).

Method of Preparation Prepare as directed under Injections, with Glucose.
No preservative is added.

Description Glucose Injection is clear, colorless liquid, and has a sweet taste. It occurs as colorless to pale yellow, clear liquid when its labeled concentration is not less than 40 %.

Identification Measure a volume of Glucose Injection, equivalent to 0.1 g of glucose according to the labeled amount and, if necessary, add water or evaporate on a water-bath to make of 2 mL. Add 2 to 3 drops of this solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

pH 3.5 ~ 6.5. In the case where the labeled concentration of the injection exceeds 5 %, dilute to 5 % with water before the test.

Purity ***5-Hydroxymethylfurfural and related substances***—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of glucose according to the labeled amount and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry: it is not more than 0.80.

Sterility Test It meets the requirement.

Baterial Endotoxins Less than 0.50 EU/mL of Glucose Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

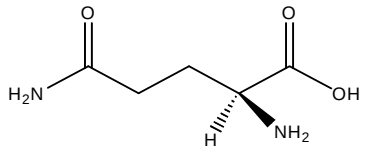
Assay Measure accurately a volume of Glucose Injection, equivalent to about 4 g of glucose ($C_6H_{12}O_6$) and add 0.2 mL of ammonia TS and water to make exactly 100 mL. Shake the solution well, allow to stand for 30 minutes and determine the optical rotation, α_D, at 20 ± 1 ºC in a 100-mm cell as directed under the

optical Rotation Determination.

Amount (mg) of glucose ($C_6H_{12}O_6$) = $\alpha_D \times 1895.4$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Glutamine



L-glutamine $C_5H_{10}N_2O_3$: 146.15

(*S*)-2,5-Diamino-5-oxopentanoic acid [*56-85-9*]

Glutamine, when dried, contains not less than 99.0 % and not more than 101.0 % of L-glutamine ($C_5H_{10}N_2O_3$).

Description Glutamine appears as white crystals or crystalline powder and has a faint, characteristic taste. Glutamine is freely soluble in formic acid, soluble in water and practically insoluble in ethanol (95).

Identification Determine the infrared spectra of Glutamine and Glutamine RS as directed in the potassium bromide disk method under the Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +6.3 ~ + 7.3° (Weigh accurately about 2 g of dried Glutamine, add 45 mL of water, warm to 40 °C to dissolve. Allow to cool, add water to make exactly 50 mL and determine the optical rotation in a 100 mm cell within 60 minutes.)

pH Dissolve 1 g of Glutamine in 50 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Glutamine in 20 mL of water: the solution is colorless and clear.

(2) ***Chloride***— Proceed with 0.5 g of Glutamine and perform the test. Use 0.30 mL of 0.01 mol/L hydrochloric acid VS as the control solution (not more than 0.021%).

(3) ***Sulfate***—Proceed with 0.6 g of Glutamine and perform the test. Use 0.35 mL of 0.005 mol/L sulfuric acid VS as the control solution (not more than 0.028%).

(4) ***Ammonium***—Proceed with 0.10 g of Glutamine and perform the test. Use 10.0 mL of ammonium standard solution as the control solution (not more than 0.1 %). Perform the test as directed under the Vacuum Distillation Method and maintain the temperature of a water-bath at 45°C.

(5) ***Heavy metals***—Proceed with 1.0 g of Glutamine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Iron***—Proceed with 1.0 g of Glutamine according to Method 1 to prepare the test solution, and perform the test according to Method A. Prepare the control solution with 1.0 mL of standard iron solution (not more than 10 ppm).

(7) ***Related substances***—Dissolve 0.10 g of Glutamine in 10 mL of water and use this solution as the test solution. Pipet 1 mL of this solution and add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm, and dry the plate at 80 °C for 30 minutes. Spray the plate evenly with a mixture of ninhydrin methanol and acetic acid (100) (97 : 3) (1 in 100) and heat the plate at 80 °C for 10 minutes: the spots other than the principal spot obtained from the test solution are not more intense than that from the standard solution (not more than 0.5 %).

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.15 g of Glutamine, add 3 mL of formic acid and 50 mL of acetic acid (100) to dissolve and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 14.615 mg of $C_5H_{10}N_2O_3$

Containers and Storage ***Containers***—Tight containers.

Glycerin

Glycerol $C_3H_8O_3$: 92.09

Propane-1,2,3-triol [*56-81-5*]

Glycerin contains not less than 84.0 % and not more than 87.0 % of glycerin ($C_3H_8O_3$).

Description Glycerin is a clear, colorless, viscous liquid**,** is odorless and has a sweet taste.

Glycerin is miscible with water or with ethanol (95).
Glycerin is hygroscopic.

Identification Proceed as directed in the Identification under Concentrated Glycerin.

Refractive index n_D^{20} : 1.449 ~ 1.454.

Specific Gravity d_{20}^{20} : 1.221 ~ 1.230.

Purity Proceed as directed in the Purity under Concentrated Glycerin.

Water 13 ~ 17 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Proceed as directed in the Residue on ignition under Concentrated Glycerin.

Assay Proceed as directed in the Assay under Concentrated Glycerin.

Containers and Storage ***Containers***—Tight containers.

Gramicidin

[*1405-97-6*]

Gramicidin is the mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus brevis* Dubos.
Gramicidin contains not less than 900 μg (potency) per mg of gramicidin, calculated on the dried basis.

Description Gramicidin appears as white to pale yellow crystalline powder.
Gramicidin is freey soluble in methanol, soluble in ethanol (99.5) and practically insoluble in water.

Identification (1) To 10 mg of Gramicidin add 2mL of 6 mol/L hydrochloric acid TS, and heat in a water bath for 30 minutes with occasional stirring. After cooling, neutralize with 6 mol/L sodium hydroxide TS, add 1 mL of ninhydrin·acetic acid TS and 0.5 mL of pyridine, and heat for 2 minutes: a blue-purple to red-purple color develops.

(2) Determine the absorption spectra of the solutions of Gramicidin and Gramicidin RS in methanol (1 in 25000), as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

Crystallinity Test It meets the requirement.

Melting Point Not less than 229 °C (after drying)

pH The pH of a solution obtained by dissolving 0.4 g of Gentamicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Loss on Drying Not more than 3.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 1.0 % (1 g).

Assay ***The Turbidimetric method*** (1) Agar medium for transferring testorganism-

Casein peptone	5.0 g		
Potassium dihydrogen phosphate			2.0 g
Yeast extract	20.0 g	Polysorbate 80	0.1 g
Glucose	10.0 g	Agar	15.0 g
Water	1000 mL		

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.7 to 6.9 after sterilization.

(2) Liquid medium for suspending test organisms- Use the culture medium in III 1 2) under Microbial Assay for Antibiotics.

(3) Test organism and preparation of the test organism suspension- Use *Enterococcus hirae* ATCC 10541 as test organism. Puncture the test organism in the agar medium for transferring test organism, subculture at least 3 times at between 36.5 °C and 37.5 °C for 20 to 24 hours and store at between 1 °C and 5 °C. Transfer the organism so obtained in 10 mL of the liquid medium for suspending test organism, incubate at between 36.5 °C and 37.5 °C for 20 to 24 hours and use this medium as the stock suspension of the test organism. Before use, add the liquid medium for suspending test organism to give 50 % to 60 % transmittance at 580 nm and to 1 mL of this suspension, add 200 mL of the liquid medium for suspending test organism, and use this as the test organism suspension.

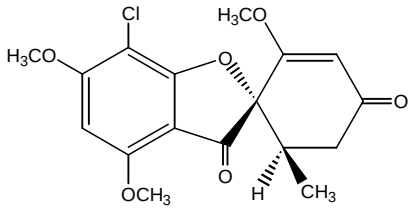
(4) Weigh accurately an amount of Gramicidin equivalent to about 10 mg (potency) and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet a suitable amount of this solution, dilute with a dilution solvent to make the test solution containing 0.02 μg (potency) per mL, and use this as the test solution. Separately, weigh accurately an amount of Gramicidin RS (previously dried for 3 hours at 60 °C and not more than 0.67 kPa) and dissolve in ethanol (99.5) to make exactly 100 mL, and use this as the standard stock solution. Keep the standard solution at not exceeding 5 °C and use within 30 days. Before use, pipet a suitable amount of this standard stock solution, dilute with a dilution solvent to obtain a solution containing 0.02 μg (potency) per mL, and use this as the standard solution. Place 0.155 mL, 0.125mL, 0.100mL, 0.080mL and 0.065mL of the standard solution, 0.100 mL of the test solution and 0.100 mL of the dilution solvent into test tubes. To each, add 10 mL of the test organism suspension, insert a stopper, incubate at 37.5 °C for 180 to 270 minutes and add 0.5 mL of formaldehyde solution (1 in 3). Perform the test according to the Turbidimetric method (III 6) as directed under Microbial Assay for Antibiot-

ics, at 530 nm.

Dilution solvent—To 390 mL of propylene glycol, add 210 mL of a mixture of ethanol (99.5) and acetone (9:1) and add distilled water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Griseofulvin



$C_{17}H_{17}ClO_6 \cdot HCl$: 352.77

(1′*S*,6′*R*)-7-Chloro-2',4,6-trimethoxy-6'-methyl-3*H*-spiro[benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione
[*126-07-8*]

Griseofulvin is a substance having antifungal activity produced by the growth of *Penicillium griseofulvum* or *Penicillium janczewskii*.
Griseofulvin contains not less than 960 μg (potency) and not more than 1020 μg (potency) per mg of griseofulvin ($C_{17}H_{17}ClO_6$), calculated on the dried basis.

Description Griseofulvin appears as white crystals or crystalline powder.
Griseofulvin is soluble in *N,N*-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Griseofulvin and Griseofulvin RS in ethanol (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Griseofulvin and Griseofulvin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers .

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +350 ~ +364° (0.25 g calculated on the dried basis, *N,N*-dimethylformamide, 25 mL, 200 mm)

Melting Point 218 ~ 222 °C.

Purity (1) ***Acidity***—Dissolve 0.25 g of Griseofulvin in 20 mL of neutralized ethanol and add 2 drops of phenolphthalein TS and 1.0 mL of 0.02 mol/L sodium hydroxide: a red color develops.

(2) ***Heavy metals***—Proceed with 1.0 g of Griseofulvin according to Method 2 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 25 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Griseofulvin according to Method 3 and perform the test (not more than 25 ppm).

(4) ***Petroleum ether solubles***—Add 1.0 g of Griseofulvin to 20 mL of petroleum ether, shake, boil for 10 minutes under a reflux condenser and cool. Filter through dry filter paper and wash the filter paper with two 15 mL volumes of petroleum ether. Combine the filtrate and washings, evaporate the petroleum ether in a water-bath and dry the residue at 105 °C for 1 hour: the weight of the residue is not more than 0.2 %.

(5) ***Related substances***—To 0.10 g of Griseofulvin, add exactly 1 mL of the internal standard solution, dissolve in acetone to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 5.0 mg of Griseofulvin RS, add 1 mL of the internal standard solution, dissolve in acetone to make 10 mL and use this solution as the standard solution. Perform the test with 2 μL of each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions. Determine each peak area by the automatic integration method and calculate the ratios, Q_1, Q_2 and Q_S, of the peak area of dechloro-griseofulvin to that of the internal standard of the test solution, the peak area of dehydrogriseofulvin to that of the internal standard of the test solution, and the peak area of Griseofulvin to that of the internal standard of the standard solution, respectively: Q_1/Q_S is not more than 0.6 (not more than 3.0 %) and Q_2/Q_S is not more than 0.15 (not more than 0.75 %).

Internal standard solution—A solution of 9,10-diphenylanthracene in aetone (1 in 500)

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 4 mm in internal diameter and about 1 m in length, packed with diatomaceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with 25 % phenyl to 25 % cyanopropyl methyl silicon polymer for gas chromatography at the ratio of 1 %.

Column temperature: A constant temperature of about 250 °C

Injection port temperature: A constant temperature of about 270 °C

Detector temperature: A constant temperature of about 300 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of Griseofulvin is about 10 minutes.

Systemic suitability

Test for required detectability: Pipet 1 mL of the standard solution and add the acetone solution (1 in 10) of the internal standard solution to make exactly 10 mL. Confirm that the ratio of the peak area of Griseofulvin to that of the internal standard obtained from 2 μL of this solution is 7 % to 13 % of the ratio of the peak area of Griseofulvin to that of the internal standard of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and Griseofulvin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of Griseofulvin to that of the internal standard is not more than 5.0 %.

Relative retention time: The retention times of dechlorogriseofulvin and dehydrogriseofulvin relative to Griseofulvin are 0.6 and 1.2, respectively.

Abnormal Toxicity Suspend and dissolve 0.1 g of Griseofulvin in 0.5 to 1 mL of distilled water and administer orally to each of 5 healthy mice weighing 17 to 22 g. Use animals in which no abnormalities are observed for at least 5 days prior to the test: no animals die during the 48 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

Loss on Drying Not more than 1.0 % (0.1 g, in vacuum not more than 0.67 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately 50 mg (potency) of each of Griseofulvin and Griseofulvin RS and dissolve in 50 mL of *N,N*-dimethylformamide. To each, add exactly 20 mL of the internal standard solution, add water to make exactly 250 mL and use these solutions as the test solution and the standard solution. Perform the test with 10 μL of each of the test solution and the standard solution as directed under Liquid Chromatography and determine the ratios of the peak area of Griseofulvin to that of the internal standard: Q_T and Q_S.

$$\text{Amount [μg (potency)] of griseofulvin } (C_{17}H_{17}ClO_6) = \textit{Amou}\text{nt [μg (potency)] of Griseofulvin RS} \times \frac{Q_T}{Q_s}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 400)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography(10 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of water and acetonitrile (3:2)

Flow rate: Adjust the flow rate so that the retention time of griseofulvin is about 6 minutes.

Systemic suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, Griseofulvin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

Systemic repeatability: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of ratios of the peak area of Griseofulvin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Griseofulvin Tablets

Griseofulvin Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of Griseofulvin ($C_{17}H_{17}ClO_6$: 352.77).

Method of Preparation Prepare as directed under Tablets, with Griseofulvin.

Identification To a quantity of powdered Griseofulvin Tablets, equivalent to 15 mg (potency) of Griseofulvin according to the labeled amount, add 100 mL of ethanol (95), shake vigorously, and filter. To 1 mL of the filtrate add ethanol (95) to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 234 nm and 238 nm, between 290 nm and 294 nm, and between 323 nm and 328 nm.

Loss on Drying Not more than 5.0 % (0.1 g (finely powdered), 0.7 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 tablet of Griseofulvin Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 1000 mL of a solution containing 40.0 mg of sodium lauryl sulfate per mL of water as the dissolution solution. Take the dissolved solution 90 minutes after the start of the test, filter through a membrane filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a solution of methanol and water (4:1) to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Griseofulvin RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution.

Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank, and determine the absorbances, A_T and A_S, at 291 nm. The dissolution rate of Griseofulvin Tablets in 90 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of Griseofulvin ($C_{17}H_{17}ClO_6$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 100000$$

C_S: Concentration of the standard solution [mg (potency)/mL]

C: Labeled amount [mg (potency)] of Griseofulvin ($C_{17}H_{17}ClO_6$) in 1 tablet

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test. Take 1 tablet of Griseofulvin Tablets, add V/5 mL of water, treat with ultrasonic waves to disintegrate the tablet, add *N,N*-dimethylformamide to make 5V/8 mL, shake vigorously for 20 minutes, add *N,N*-dimethylformamide to make exactly V mL so that each mL contains 1.25 mg (potency) of Griseofulvin, and centrifuge. Pipet 8 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make 100 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Then, proceed as directed under the Assay.

Amount [mg (potency)] of Griseofulvin ($C_{17}H_{17}ClO_6$)

$$= W_S \times \frac{Q_T}{Q_S} \times \frac{V}{32}$$

W_S: Amount [mg (potency)] of Griseofulvin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 2000)

Assay Weigh accurately not less than 20 Griseofulvin Tablets, and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g (potency) of Griseofulvin, add water and sonicate. Add 100 mL of *N,N*-dimethylformamide, shake vigorously for 20 minutes, and add *N,N*-dimethylformamide to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make exactly 100 mL, filter through a membrane filter with a pore size of not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately an amount of Griseofulvin RS, equivalent to about 40 mg (potency), and dissolve in *N,N*-dimethylformamide to make exactly 20 mL. Pipet 5 mL of this solution, add 20 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test as directed in the Assay under Griseofulvin.

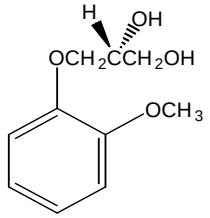
Amount [μg (potency)] of Griseofulvin ($C_{17}H_{17}ClO_6$)
= Amount [μg (potency)] of Griseofulvin RS

$$\times \frac{Q_T}{Q_S} \times \frac{25}{2}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 2000)

Containers and Storage ***Containers***—Tight containers.

Guaifenesin



and enantiomer

Guaiacol Glyceryl Ether $C_{10}H_{14}O_4$: 198.22

(*RS*)-3-(2-Methoxyphenoxy)propane-1,2-diol [*93-14-1*]

Guaifenesin, when dried, contains not less than 98.0 % and not more than 102.0 % of guaifenesin ($C_{10}H_{14}O_4$).

Description Guaifenesin appears as white crystals or crystalline powder.
Guaifenesin is freely soluble in hot water or in ethanol (95), soluble in chloroform, sparingly soluble in water and slightly soluble in ether.
A solution of ethanol (95) (1 in 20) shows no optical rotation.

Identification (1) Take 5 mL of Guaifenesin, add 1 mL of formalin-sulfuric acid TS: a red-purple color is observed.

(2) Determine the absorption spectra of solutions of Guifenesin and Guaifenesin RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Guaifenesin and Guaifenesin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 80 ~ 83 °C.

pH Dissolve 1.0 g of Guaifenesin in 100 mL of water: the pH of the solution is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Guaifenesin in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.7 g of Guaifenesin in 25 mL of water by warming. Cool, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.020 %).

(3) ***Heavy metals***—Dissolve 2.0 g of Guaifenesin in 25 mL of water by warming. Cool, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Guaifenesin according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Free guaiacol***—Take 1.0 g of Guaifenesin, add exactly 25 mL of water, dissolve by warming, cool and use this solution as the test solution. Separately, dissolve 0.10 g of guaiacol in water to make exactly 1000 mL. Pipet 3.0 mL of this solution, add exactly 22 mL of water and use this solution as the standard solution. To each of the test solution and the standard solution, add 1.0 mL of potassium ferricyanide TS and 5.0 mL of a solution of 4-aminoantipyrine (1 in 200) and immediately after shaking for exactly 5 seconds, add a solution of sodium bicarbonate (1 in 1200) to make exactly 100 mL. Determine the absorbances of the test solution and the standard solution at 500 nm exactly 15 minutes after the addition of the 4-aminoantipyrin solution as directed under Ultraviolet-visible Spectrophotometry, using a solution, prepared in the same manner with 25 mL of water, as the blank: the absorbance of the solution obtained from the test solution is not greater than that from the standard solution.

(6) ***Related substances***—Dissolve 1.0 g of Guaifenesin in 100 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Develop the plate with a mixture of ether, ethanol (95) and ammonia solution (28) (40 : 10 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly *p*-dimethylaminobenzaldehyde TS for spraying on the plate and heat at 110 ºC for 10 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

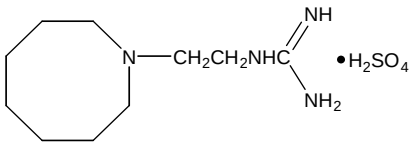
Assay Weigh accurately about 60 mg of each of Guaifenesin and Guaifenesin RS, previously dried, and dissolve in water to make exactly 100 mL. Pipet 5 mL of each of these solutions, add water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbance, A_T and A_S, of these solutions at 273 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of guaifenesin } (C_{10}H_{14}O_4) = \frac{A_T}{A_S} \times W_S$$

W_S: Amount (mg) of Guaifenesin RS

Containers and Storage ***Containers***—Tight containers.

Guanethidine Sulfate



$C_{10}H_{22}N_4H_2SO_4$: 296.39

2-[2-(Azocan-1-yl)ethyl]guanidine;sulfuric acid
[*645-43-2*]

Guanethidine Sulfate, when dried, contains not less than 98.5 % and not more than 101.0 % of guanethidine sulfate ($C_{10}H_{22}N_4 \cdot H_2SO_4$).

Melting point—251 ~ 256 (an evacuated sealed capillary tube, with decomposition).

Identification (1) Take 4 mL of a solution of Guanethidine Sulfate (1 in 4000), add 2 mL of 1-naphthol TS, 1 mL of diacetyl TS and 15 mL of water and allow to stand for 30 minutes: a red color is observed.

(2) Determine the infrared spectra of Guanethidine Sulfate and Guanethidine Sulfate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers .

(3) A solution of Guanethidine Sulfate (1 in 10) responds to the Qualitative Tests for sulfate.

pH Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the pH of the solution is between 4.7 and 5.7.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Guanethidine Sulfate according to Method 4 and per-

form the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Methylisothiourea sulfate***—Dissolve 2.0 g of Guanethidine Sulfate in 80 mL of sodium hydroxide TS and allow to stand for 10 minutes. Add 60 mL of hydrochloric acid, 2 g of sodium bromide and water to make 200 mL. Then, to this solution, add 0.70 mL of 1/60 mol/L potassium bromate VS and 2 mL of zinc iodide-starch paste TS: a blue color is observed.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

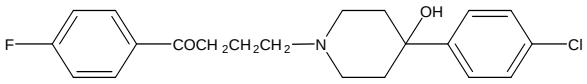
Assay Weigh accurately about 0.5 g of Guanethidine Sulfate, previously dried, dissolve in 100 mL of formic acid and add 70 mL of a mixture of acetic anhydride and glacial acetic acid (100) (6 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.639 mg of $C_{10}H_{22}N_4 \cdot H_2SO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Haloperidol



$C_{21}H_{23}ClFNO_2$: 375.86

4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one [*151-67-7*]

Haloperidol, when dried, contains not less than 99.0 % and not more than 101.0 % of haloperidol ($C_{21}H_{23}ClFNO_2$).

Description Haloperidol appears as white to pale yellow crystals or powder.
Haloperidol is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in 2-propanol or in ethanol (99.5) and practically insoluble in water.

Identification (1) Dissolve 30 mg each of Haloperidol and Haloperidol RS in 100 mL of 2-propanol. Add 10 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol in 5 mL of these solutions to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Haloperidol and Haloperidol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 149 ~ 153 ºC.

Purity (1) ***Sulfate***—Take 1.0 g of Haloperidol, add 50 mL of water, shake and filter. Take 25 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 5 mmol/L sulfuric acid VS (not more than 0.048 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Haloperidol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 25 mg of Haloperidol in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 µL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of peak other than haloperidol from the test solution is not larger than 2 times the peak area of haloperidol from the standard solution. Use the peak areas, obtained by the automatic integration method, having the relative retention time of about 0.15, 1.2 and 2.6 with respect to haloperidol, after multiplying their relative response factors, 0.75, 1.47 and 0.76, respectively.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsylanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature at about 40 ºC.

Mobile phase: Dissolve 2.95 g of sodium citrate in 900 mL of water, add dilute hydrochloric acid to adjust pH 3.3, add water to make 1000 mL. Add 700 mL of methanol in 300 mL of this solution, add 1 g of sodium lauryl sulfate, and dissolve.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

System suitability

Test for required detection: Pipet 5 mL of standard solution, and add the mobile phase to make 25 mL. The peak area of haloperidol obtained from 10 µL of this solution is between 15 and 25 % of the peak area of haloperidol obtained from the standard solution.

System performance: Perform the test with 10 uL of the standard solution according to the above operating conditions: the number of theoretical plates and symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 uL each of the standard solution according to the above operating conditions, the relative standard deviation of haloperidol peak area is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of haloperidol after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 ºC, P_2O_5, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

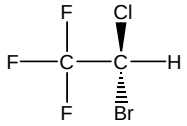
Assay Weigh accurately about 0.6 g of Haloperidol, previously dried and dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 1 drop of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.587 mg of $C_{21}H_{23}ClFNO_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Halothane



and enantiomer

$C_2HBrClF_3$: 197.38

2-Bromo-2-chloro-1,1,1-trifluoroethane [*151-67-7*]

Halothane contains not less than 0.008 % and not more than 0.012 % of Thymol as a stabilizer.

Description Halothane appears as clear, colorless and mobile liquid.

Halothane is miscible with ethanol (95), with ether or with isooctane.

Halothane is slightly soluble in water.

Halothane is a volatile, nonflammable liquid and setting fire to its heated vapor does not support combustion.

Halothane is affected by light.

Refractive index— n_D^{20} : 1.369 ~ 1.371.

Identification Transfer about 3 μL each of Halothane and Halothane RS to gas cells having light path, 10 cm in length and determine the infrared spectra as directed in the gas sampling method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Gravity d_{20}^{20} : 1.872 ~ 1.877.

Purity (1) ***Acid or alkali***—Shake 60 mL of Halothane with 60 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer and use this solution as the test solution. To 20 mL of the test solution, add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color is observed. To 20 mL of the test solution, add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is observed.

(2) ***Halide and halogen***—To 5 mL of the test solution obtained in (1), add 1 drop of nitric acid and 0.20 mL of silver nitrate TS: no turbidity is produced. To 10 mL of the test solution obtained in (1), add 1 mL of potassium iodide TS and 2 drops of starch TS and allow to stand for 5 minutes: a blue color is not observed.

(3) ***Phosgene***—Transfer 50 mL of Halothane to a dried conical flask, suspend a strip of phosgene test paper vertically inside the flask with the lower end about 10 mm above the surface of the liquid, insert the stopper and allow to stand in a dark place for 20 to 24 hours: the test paper shows no yellow color.

(4) ***Residue on evaporation***—Pipet 50 mL of Halothane, evaporate on a water-bath and dry the residue at 105 ºC for 2 hours: the amount of the residue is not more than 1.0 mg.

(5) ***Volatile related substances***—Take 100 mL of Halothane, add exactly 5.0 μL of the internal standard and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Gas Chromatography according to the following conditions and determine each peak area by the automatic integration method: the total area of the peaks other than those of Halothane and the internal standard is not larger than the peak area of the internal standard.

Internal standard substance—1,1,2-trichloro-1,2,2-trifluoro-ethane.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and 3 m in length, the first 2 m from the injection port packed with siliceous earth for gas chromatography (180 μm to 250 μm in particle diameter), coated with macrogol 400 at the ratio of 30 %, and the remaining 1 m packed with siliceous earth for gas chromatography (180 μm to 250 μm in particle diameter), coated with dinonyl phthalate at the ratio of 30 %.

Column temperature: A constant temperature of about 50 ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is 2 minutes to 3 minutes.

System suitability

System performance: Mix 3 mL of Halothane and 1 mL of the internal standard. When the procedure is run with 1 μL of this solution, as directed under the above operating conditions, the internal standard and Halothane are eluted in this order with a resolution between their peaks being not less than 10.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the internal standard obtained from 5 μL of the test solution composes 30 % to 70 % of the full scale.

Time span of measurement: About 3 times as long as the retention time of Halothane.

Distilling Range Not less than 95 vol % distils within a 1 ºC range between 49 ºC and 51 ºC.

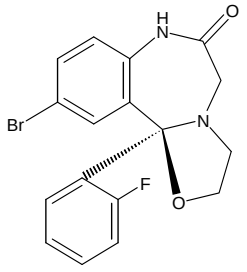
Thymol To 0.50 mL of Halothane, add 5.0 mL of isooctane and 5.0 mL of titanium (IV) dioxide TS, shake vigorously for 30 seconds and allow to stand: the separated upper layer has more color than the following control solution A and has no more color than the following control solution B.

Control solution—Dissolve 0.225 g of Thymol RS in isooctane to make exactly 100 mL. Take 10 mL each of this solution, accurately measured, add isooctane to make exactly 150 mL and 100 mL, respectively. Proceed with 0.50 mL each of these solutions in the same manner as Halothane and use the separated upper layers obtained from the control solutions A and B, respectively.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 30 ºC.

Haloxazolam



and enantiomer

$C_{17}H_{14}BrFN_2O_2$: 377.21

10-Bromo-11*b*-(2-fluorophenyl)-2,3,7,11*b*-tetrahydrobenzo[f]oxazolo[3,2-d][1,4]diazepin-6(5*H*)-one [*59128-97-1*]

Haloxazolam, when dried, contains not less than 99.0 % and not more than 101.0 % of haloxazolam ($C_{17}H_{14}BrFN_2O_2$).

Description Haloxazolam appears as white crystals or crystalline powder and is odorless and tasteless.
Haloxazolam is freely soluble in acetic acid (100), sparingly soluble in acetonitrile, in methanol or in dehydrated ethanol, slightly soluble in ether and practically insoluble in water.

Melting point—About 183 ºC (with decomposition).

Identification (1) Dissolve 10 mg of Haloxazolam in 10 mL of methanol, add 1 drop of hydrochloric acid under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. To this solution, add 1 mL of sodium hydroxide TS: the fluorescence disappears immediately.

(2) Prepare the test solution with 50 mg of Haloxazolam as directed under the Oxygen Flask Combustion Method, using a mixture of 20 mL of dilute sodium hydroxide TS and 1 mL of strong hydrogen peroxide water as an absorbing liquid: the test solution responds to the Qualitative Tests for bromide and for fluoride.

(3) Determine the absorption spectra of solutions of Haloxazolam and Haloxazolam RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Haloxazolam and Haloxazolam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Absorbance $E_{1\,cm}^{1\%}$ (247 nm) 390 ~ 410 (10 mg, methanol, 1000 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Haloxazolam in 20 mL of dehydrated ethanol: the solution is clear and colorless.

(2) ***Soluble halides***—Take 1.0 g of Haloxazolam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and use this solution as the test solution. Perform the test with this solution as directed under the Chloride Limit Test. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid (Cl: not more than 0.0071 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Haloxazolam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Take 1.0 g of Haloxazolam in a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, repeat this procedure twice, add several 2 mL of strong hydrogen peroxide water and heat until the solution is colorless

to pale yellow. After cooling, add 2 mL of a saturated solution of ammonium oxalate and heat until white fumes are evolved. After cooling, add water to make 5 mL and perform the test with this solution: the solution has no more color than the following control solution (not more than 2 ppm).

Control solution—Proceed in the same manner as above without using Haloxazolam, add 2.0 mL of standard arsenic solution and water to make 5 mL and proceed in the same manner as the test solution.

(5) ***Related substances***—Dissolve 0.10 g of Haloxazolam in 100 mL of acetonitrile and use this solution as the test solution. Pipet 1 mL of the test solution, add acetonitrile to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of haloxazolam from the test solution is not larger than the peak area of haloxazolam from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 6.2 g of boric acid and 7.5 g of potassium chloride in 900 mL of water, adjust the pH with triethylamine to 8.5 and add water to make 1000 mL. To 3 volumes of this solution, add 2 volumes of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of galoxazolam is about 10 minutes.

System suitability

Test for required detection: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 50 mL. Confirm that the peak area of haloxazolam obtained from 10 μL of this solution is between 8 and 12 % of the peak area of haloxazolam obtained from the standard solution.

System performance: Dissolve 10 mg each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. When the precedure is run with 10 μL of this solutions, as directed under the above operating conditions, haloxazolam and cloxazolam are eluted in this order with a resolution between their peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of standard solution according to the above conditions: the relative deviation of the peak area of haloxazolam is not more than 1.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

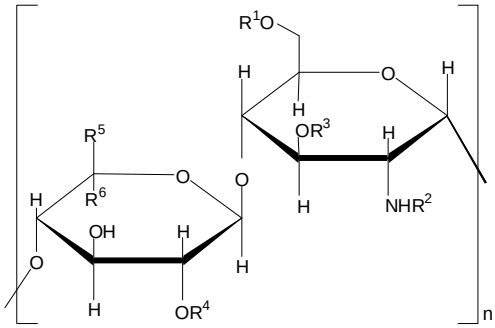
Assay Weigh accurately about 0.5 g of Haloxazolam, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.721 mg of $C_{17}H_{14}BrFN_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Heparin Sodium



R^1, R^3, R^4 = SO_3Na or H

R^2 = SO_3Na or 

R^5 = CO_2Na, R^6 = H or R^5 = H, R^6 = CO_2Na

Sodum (3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*)-4,6-dihydroxy-5-(sulfonatoamino)-2-((sulfonatooxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-dihydroxy-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate [*9041-08-1*]

Heparin Sodium is obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals and prolongs the clotting time of blood.

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid). Plasma proteins antithrombin and heparin cofactor II form polymers, inactivating thrombin (blood coagulation factor IIa) and prolonging the clotting time of blood. Other coagulation factors, such as activated blood coagulation factor X (blood coagulation factor Xa), are also inhibited. The ratio of the inhibitory potency of blood coagulation factor Xa to that of blood coagulation factor IIa is between 0.9 and 1.1.

Heparin Sodium contains not less than 180 heparin units (IU) per mg, calculated on the dried basis.
Label the name of the organ and animal species used as the starting material.

Description Heparin Sodium is white to grayish brown powder or grains and is odorless.
Heparin Sodium is soluble in water and practically insoluble in ethanol (95) or in ether.
Heparin Sodium is hygroscopic.

Identification (1) Proceed as directed in the Assay: it meets the requirement.

(2) Dissolve separately 20 mg each of Heparin Sodium and Heparin Sodium RS in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-*d*4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10000), and use these solutions as the test solution and standard solution, respectively. Determine the ^{1}H spectra of the test solution and standard solution as directed under Nuclear Magnetic Resonance Spectroscopy according to the operating conditions in the Purity (6), using sodium 3-trimethylsilylpropionate-*d*4 for nuclear magnetic resonance spectroscopy as an internal reference compound: the test solution and standard solution exhibit signals of similar area intensity at δ 2.03 to 2.07 ppm, at δ 3.25 to 3.31 ppm, at δ 5.20 to 5.26 ppm, and at δ 5.39 to 5.45 ppm.

(3) Dissolve separately 1 mg each of Heparin Sodium and Heparin Sodium RS in 1 mL of water, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the retention time of the principal peak obtained from the test solution corresponds to that from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 202 nm)

Column: A stainless steel column 2.0 mm in internal diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-3	90	10
3-15	90→0	10→100

Flow rate: 0.2 mL/minute

System suitability

System performance: Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water, and use this solution as the dermatan sulfate standard solution. Dissolve 1.0 mg of Heparin Sodium RS in 0.60 mL of water, and use this solution as the heparin sodium standard solution. Mix well 90 μL of the heparin sodium standard solution, 30 μL of the over-sulfated chondroitin sulfate standard solution, and 30 μL of the dermatan sulfate standard solution, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, dermatan sulfate, heparin, and over-sulfated chondroitin sulfate are eluted in this order with the resolutions between the peaks of dermatan sulfate and heparin and between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.0 and not less than 1.5, respectively.

(4) ***Ratio of the inhibitory potency of blood coagulation factor Xa to that of blood coagulation factor IIa*** *Inhibitory potency of blood coagulation factor Xa*

(i) Substrate solution: Dissolve *N*-benzoyl-*L*-isoleucyl-*L*-glutamyl(γ-OR)-glycyl-*L*-arginine-*p*-nitrianilide hydrochloride in water to render a concentration of 1 mmol/L.

(ii) Antithrombin solution: Dissolve human antithrombin III in pH 8.4 buffer solution so that each mL contains 1.0 IU of antithrombin.

(iii) Blood coagulation factor Xa solution: Dissolve bovine blood coagulation factor Xa in pH 8.4 buffer solution to make a solution so that the absorbance, determined at 405 nm as directed in the Assay using 30 μL of pH 8.4 buffer solution instead of the standard solution or test solution, is between 0.65 and 1.25.

(iv) pH 8.4 buffer solution: Dissolve 2-amino-2-hydroxymethyl-1,3-propandiol, disodium ethylenediaminetetraacetate dihydrate, and sodium chloride in water containing 0.1 % polyethylene glycol 6000 to render the concentrations of 0.050 mol/L, 0.0075 mol/L, and 0.175 mol/L, respectively. If necessary, adjust the pH to 8.4 with hydrochloric acid or sodium hydroxide solution.

(v) Reaction stop solution: To 20 mL of acetic acid (100) add water to make 100 mL.

(vi) Heparin standard solutions- Dissolve Heparin Sodium RS in a suitable amount of water, and use this solution as the standard stock solution. Pipet a volume of this solution, add pH 8.4 buffer solution to obtain at least 5 dilutions in the concentration range between

0.03 to 0.375 unit/mL, and use these solutions as the standard solutions. Prepare at least 2 solutions of each concentration.

(vii) Test solutions: Take exactly a suitable amount of Heparin Sodium according to the labeled units, dissolve in pH 8.4 buffer solution to make the same concentrations as the standard solutions, and use these solutions as the test solutions. Prepare at least 2 solutions of each concentration.

(viii) Procedure: To each test tube, previously warmed in a tank at 37 °C, add 120 μL of pH 8.4 buffer solution. Add 30 μL each of the test solutions and standard solutions to a test tube, respectively, add 150 μL each of the antithrombin solution, previously warmed at 37 °C for 15 minutes, mix, and allow to stand for 2 minutes. To each solution add 300 μL each of the blood coagulation factor Xa solution, previously warmed at 37 °C for 15 minutes, mix, and allow to stand for 2 minutes. Add 300 μL each of the substrate solution, previously warmed at 37 °C for 15 minutes, mix, and allow to stand for 2 minutes. To each solution add 150 μL each of the reaction stop solution, and shake each test tube. Determine the absorbances at 405 nm of the test solutions and standard solutions as directed under Ultraviolet-visible Spectrophotometry, using the following solution as the blank: transfer 150 μL of the reaction stop solution to a test tube, and mix with the above solutions in reverse order, respectively.

(ix) Calculation: Plot a graph of the log absorbances on the vertical axis and the concentration of heparin, calculated on the dried basis, in each heparin standard solution or test solution on the horizontal axis. Calculate the inhibitory potency of blood coagulation factor Xa in Heparin Sodium by the following equation. The ratio (r) of the inhibitory potency of blood coagulation factor Xa to that of blood coagulation factor IIa as obtained in the Assay is between 0.9 and 1.1.

$$\text{Inhibitory potency (heparin unit/mg) of blood coagulation factor Xa in Heparin Sodium} = \text{Inhibitory potency (heparin unit/mg) of Heparin Sodium RS} \times \frac{S_T}{S_S}$$

S_T: Gradient of the test solution
S_S: Gradient of the standard solution

$$r = \frac{\text{Inhibitory potency (heparin unit/mg) of blood coagulation factor Xa}}{\text{Inhibitory potency (heparin unit/mg) of blood coagulation factor IIa}}$$

(5) Heparin Sodium responds to the Qualitative Tests (1) for sodium salt.

pH The pH of a solution of 1.0 g of Heparin Sodium in 100 mL of water is between 5.0 and 7.5.

Purity (1) ***Clarity and color of solution*—**Dissolve 0.5 g of Heparin Sodium in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Heparin Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Barium*—**Dissolve 30 mg of Heparin Sodium in 3.0 mL of water and use this solution as the test solution. To 1.0 mL of the test solution, add 3 drops of dilute sulfuric acid and allow to stand for 10 minutes: no turbidity is produced.

(4) ***Total nitrogen*—**Weigh accurately about 100 mg of Heparin Sodium, previously dried at 60 ºC for 3 hours under reduced pressure and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 1.3 % and not more than 2.5 %.

(5) ***Protein***—Weigh accurately a suitable amount of Heparin Sodium, dissolve in water so that each mL contains 5 mg, and use this solution as the test solution (prepare 3 identical solutions and use these solutions as the test solutions (1), (2), and (3)). Separately, weigh accurately a suitable amount of bovine serum albumin, dissolve in water so that each mL contains 0.100 mg, dilute a suitable volume of this solution with water to make at least 5 dilutions in the concentration range between 0.005 and 0.100 mg/mL, and use these solutions as the standard solutions. To 1 mL each of the standard solutions, test solutions (1), (2), and (3), and water (blank solution) add 5 mL each of the reaction solution, and allow to stand at room temperature for 10 minutes. To these solutions add 0.5 mL each of diluted Folin's TS, mix immediately, and allow to stand at room temperature for 30 minutes. Determine the absorbances at 750 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank, plot a calibration curve with the absorbances of the standard solution, and calculate the content of protein in the test solution using the calibration curve (not more than 1.0 %).

Reaction solution—Weigh accurately a suitable amount of sodium hydroxide, and dissolve in water so that each 1000 mL contains 10 g. Separately, dissolve sodium carbonate decahydrate in water so that each 1000 mL contains 50 g. Mix equal volumes of the two solutions, mix 4 volumes of this solution with 1 volume of water, and use this solution as the solution A. Dissolve a suitable amount of sodium tartrate dihydrate in water so that each 1000 mL contains 29.8 g. Separately, dissolve a suitable amount of copper sulfate in water so that each 1000 mL contains 12.5 g. Mix equal volumes of these two solutions, mix 4 volumes of this solution with 1 volume of water, and use this solution as the solution B. Mix 50 volumes of the solution A with 1 volume of the solution B.

Diluted Folin's TS—Dilute Folin's TS 2 to 4 times with water so that the pH of the test solutions and standard solutions after addition of the reagent and Folin's TS is 10.25 ± 0.25 in the above procedure.

(6) ***Over-sulfated chondroitin sulfate*** (i) *Nuclear magnetic resonance spectroscopy*—Dissolve 20 mg each of Heparin Sodium and Heparin Sodium RS in a 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-*d*4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 1000), and use these solutions as the test solution and standard solution. Determine the ^{1}H spectrum of the test solution and standard solution as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropionate-*d*4 as an internal reference compound: the signal of over-sulfated chondroitin sulfate is not observed between δ 2.13 and 2.17 ppm (the hydrogen signal of the *N*-acetyl groups of over-sulfated chondroitin sulfate is observed between 2.13 and 2.17 ppm).

Operating conditions

Instrument: ^{1}H-pulse Fourier transform nuclear magnetic resonance spectrometer

Frequency: Not less than 400 MHz

Temperature: 25 °C

Spectrum range: DHO signal ± 6.0 ppm

Pulse angle: 90 °

Pulse repetition time: 20 seconds

Repeat scan: 4 times

Signal-to-noise ratio: The signal-to-noise ratio at the hydrogen signal (around 2 ppm) of the *N*-acetyl groups of heparin is not less than 1000.

Window function: Exponential function (line broadening factor: 0.2 Hz)

System suitability

Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-*d*4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10000), and use this solution as the over-sulfated chondroitin sulfate standard solution. Separately, dissolve 20 mg of Heparin Sodium RS in 0.40 mL of heavy water solution for nuclear magnetic resonance spectroscopy, add 0.20 mL of the over-sulfated chondroitin sulfate standard solution, and use this solution as the system suitability solution. When the procedure is run with this solution under the above operating conditions, the signals of the *N*-acetyl groups of heparin and the *N*-acetyl groups of over-sulfated chondroitin sulfate appear between δ 2.02 and 2.06 ppm and between δ 2.13 and 2.17 ppm, respectively.

(ii) *Liquid chromatography*—Proceed as directed in the Identification (3): the peak of over-sulfated chondroitin sulfate is not observed.

(7) ***Galactosamine***—Weigh accurately about 2.4 mg of Heparin Sodium, dissolve in 1.0 mL of diluted hydrochloric acid (5 in 12), and use this solution as the test stock solution. Separately, weigh accurately 8.0 mg each of *D*-glucosamine hydrochloride and *D*-galactosamine hydrochloride, dissolve separately in diluted hydrochloric acid (5 in 12) to make exactly 10 mL, and use these solutions as the glucosamine standard solution and galactosamine standard solution. To 99 volumes of the glucosamine standard solution add 1 volume of the galactosamine standard solution, and use this solution as the standard stock solution. Transfer 500 μL each of the test stock solution and standard stock solution to separate stoppered test tubes, insert the stoppers, and heat at 100 °C for 6 hours. Cool to room temperature, take 100 μL each of the solutions, and dry in vacuum. To each residue add 50 μL of methanol, dry in vacuum at room temperature, and dissolve each residue in 10 μL of water. Add 40 μL each of aminobenzoic acid derivatization TS, mix well, and heat at 80 °C for 1 hour. Cool the solutions to room temperature, and dry in vacuum. To each residue add 200 μL each of water and ethyl acetate, shake vigorously, and centrifuge for 1 minute. Discard the upper layer, to each lower layer add 200 μL of ethyl acetate, shake vigorously, and centrifuge for 1 minute. Use the lower layers so obtained as the test solution and standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the ratio of the peak area of galactosamine to that of glucosamine from the test solution is not larger than that from the standard solution.

Operating conditions

Detector: A spectrofluorometer (excitation wavelength: 305 nm, fluorescence wavelength: 360 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C

Mobile phase: To 100 mL of a mixture of water and trifluoroacetic acid (1000 : 1) add 100 mL of acetonitrile. To 140 mL of this solution add 860 mL of a mixture of water and trifluoroacetic acid (1000 : 1).

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Dissolve 8.0 mg of D-mannosamine hydrochloride in 10 mL of diluted hydrochloric acid (5 in 12), and use this solution as the mannosamine standard solution. Transfer 500 μL of a mixture of the standard stock solution and mannosamine standard solution (100 : 1) to a stoppered test tube, insert the stopper, heat at 100 °C for 6 hours, cool to room temperature, take 100 μL of this solution, and dry in vacuum. To the residue add 50 μL of methanol, dry in vacuum at room temperature, dissolve the residue in 10 μL of water, add 40 μL of aminobenzoic acid derivatization TS, and mix well. Heat this solution at 80 °C for 1 hour, cool to room temperature, and dry in vacuum. To the residue add 200 μL each of water and ethyl acetate, shake vigorously, and centrifuge for 1 minute. Discard the upper layer, to the lower layer

add 200 μL of ethyl acetate, shake vigorously, centrifuge for 1 minute, and use the lower layer so obtained as the system suitability solution. Confirm that the ratio of the peak area of galactosamine to that of glucosamine obtained from 5 μL of this solution is equivalent to 0.7 to 2.0 %.

System performance: When the procedure is run with 5 μL of the system suitability solution under the above operating conditions, glucosamine, mannosamine, and galactosamine are eluted in this order with the resolutions between the peaks of glucosamine and mannosamine and between mannosamine and galactosamine being not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of galactosamine to that of glucosamine is not more than 4.0 %.

Time span of measurement: 50 minutes

(8) ***Hexane related substances***—Weigh accurately 40 mg of Heparin Sodium, dissolve in 10 mL of water, and determine the absorbance at 260 nm of this solution as directed under Ultraviolet-visible Spectrophotometry: not more than 0.20.

Loss on Drying Not more than 5.0 % (in vacuum, 60 ºC, 3 hours).

Residue on Ignition 28.0 ~ 41.0 % (after drying, 20 mg).

Bacterial Endotoxins Less than 0.03 EU/heparin unit.

Sterility Test It meets the requirement, when Heparin Sodium is used in a sterile preparation.

Assay ***Inhibitory potency of blood coagulation factor IIa*** (i) Substrate solution: Dissolve *H-D*-phenylalanyl-*L*-pipecolyl-*L*-arginine-*p*-nitroanilide dihydrochloride in water to make 1.25 mmol/L.

(ii) Antithrombin solution: Dissolve human antithrombin III in a suitable volume of water so that each mL contains 5 IU of antithrombin. To a volume of this solution add pH 8.4 buffer solution so that each mL contains 0.125 IU of antithrombin.

(iii) Human thrombin solution: To human thrombin (blood coagulation factor IIa) add water so that each mL contains 20 IU of thrombin. To a volume of this solution add pH 8.4 buffer solution so that each mL contains 5 IU of thrombin, and use this solution as the human thrombin solution.

(iv) pH 8.4 buffer solution: Dissolve 6.10 g of 2-amino-2-hydroxymethyl-1,3-propandiol, 2.80 g of disodium ethylenediaminetetraacetate dihydrate, 10.20 of sodium chloride, not more than 10.00 g of polyethylene glycol 6000, and (or) 2.00 g of bovine serum albumin in 800 mL of water. Adjust the pH to 8.4 with hydrochloric acid, and add water to make 1000 mL.

(v) Reaction stop solution: To 20 mL of acetic acid (100) add water to make 100 mL.

(vi) Heparin standard solution: Dissolve Heparin Sodium RS in a suitable amount of water, and use this solution as the standard stock solution. Pipet an amount of this solution, add pH 8.4 buffer solution to make at least 4 dilutions in the concentration range between 0.005 and 0.03 units, and use these solutions as the standard solution. Prepare at least 2 solutions of each concentration.

(vii) Test solution: Dissolve a suitable amount of Heparin Sodium according to the labeled units in pH 8.4 buffer solution to make the same concentrations as the standard solutions, and use these solutions as the test solutions. Prepare at least 2 solutions of each concentration.

(viii) Procedure: To each test tube, previously warmed in a tank at 37 °C, add a volume (50 to 100 μL) each of the pH 8.4 buffer solution (blank solution), test solutions, and standard solutions, previously warmed at 37 °C, respectively. To each solution add twice the volume of antithrombin solution (100 to 200 μL), previously warmed at 37 °C, mix carefully without allowing bubbles to form, allow to stand at 37 °C for at least 1 minute, add 25 to 50 μL each of human thrombin solution, previously warmed at 37 °C, and allow to stand for at least 1 minute. To each solution add 50 to 100 μL of the substrate solution, previously warmed at 37 °C, allow to stand for at least 1 minute, add 50 to 100 μL of the reaction stop solution, shake, and stop the test after at least 1 minute. Determine the absorbances at 405 nm of the test solutions and standard solutions as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank. Prepare an appropriate number of blank solutions, and determine the absorbance of each blank solution immediately before at least each dilution series of test solutions and standard solutions. The relative standard deviation of the measurements of the blank solutions is not more than 10 %.

(ix) Calculation: Plot a graph of the log absorbances on the vertical axis and the concentration of heparin, calculated on the dried basis, in each heparin standard solution or test solution on the horizontal axis. Calculate the heparin units (IU) per mg of Heparin Sodium by the following equation.

$$\text{Heparin units in each mg of Heparin Sodium} = \text{Amount [heparin unit/mg (potency)] of Heparin Sodium RS} \times \frac{S_T}{S_S}$$

S_T: Gradient of the test solution
S_S: Gradient of the standard solution

Containers and Storage ***Containers***—Tight containers.

Heparin Sodium Injection

Heparin Sodium Injection is an aqueous solution for injection. Heparin Sodium Injection contains not less than 90.0 % and not more than 110.0 % of the labeled heparin units.
Label the name of the organ and animal species used as the starting material of Heparin Sodium supplied for preparing Heparin Sodium Injection.

Method of Preparation Dissolve Heparin Sodium in Isotonic Sodium Chloride Injection and prepare as directed under Injections.

Description Heparin Sodium Injection is a clear, colorless to pale yellow liquid.

pH 5.5 ~ 8.0.

Purity (1) ***Barium***—Measure exactly a volume of Heparin Sodium Injection, equivalent to 3000 units of Heparin Sodium according to the labeled unit. Add water to make 3.0 mL and use this solution as the test solution. To 1.0 mL of the test solution, add 3 drops of dilute sulfuric acid and allow to stand for 10 minutes: no turbidity is produced.

(2) ***Protein***—Proceed as directed in the Purity (5) under Heparin Sodium.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.0030 EU/heparin unit.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Proceed as directed in the Assay under Heparin Sodium, replacing the test solution indicated in (vii) and the calculation in (ix) with the following.

(vii) Test solution: Pipet a suitable volume of Heparin Sodium Injection according to the labeled units, add pH 8.4 buffer solution to render the same concentration as the standard stock solution, and use this solution as the test stock solution. Pipet a suitable volume each of this solution, dilute with pH 8.4 buffer solution to make the same concentrations as each standard solution, and use these solutions as the test solutions. Prepare at least 2 solutions of each concentration.

(ix) Calculation: Plot a graph of the log absorbances on the vertical axis and the concentration of heparin, calculated on the dried basis, in each heparin standard solution or test solution on the horizontal axis. Calculate the heparin units (IU) per mL of Heparin Sodium Injection by the following equation.

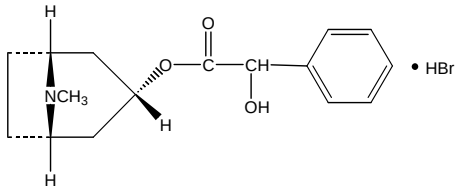
$$\text{Heparin units in each mL of Heparin Sodium Injection} = \text{Concentration (heparin unit/mL) of the standard stock solution} \times \frac{S_T}{S_S} \times \frac{b}{a}$$

S_T: Gradient of the test solution
S_S: Gradient of the standard solution
a: Amount (mL) of Heparin Sodium Injection taken
b: Total volume (mL) of the test stock solution when prepared

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Homatropine Hydrobromide



$C_{16}H_{21}NO_3 \cdot HBr$: 356.26

(1*R*,3*R*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-hydroxy-2-phenylacetate hydrobromide [*51-56-9*]

Homatropine Hydrobromide contains not less than 99.0 % and not more than 101.0 % of homatropine hydrobromide ($C_{l6}H_{21}NO_3$.HBr) calculated on the dried basis.

Description Homatropine Hydrobromide appears as white crystals or crystalline powder and is odorless.
Homatropine is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), very slightly soluble in acetic anhydride and practically insoluble in ether.
Homatropine Hydrobromide is affected by light.
Melting point—About 214 ºC (with decomposition).

Identification (1) Take 5 mL of a solution of Homatropine Hydrobromide (1 in 20), add 2 to 3 drops of iodine TS: a brown precipitate is produced.

(2) Dissolve 50 mg of Homatropine Hydrobromide in 5 mL of water and add 3 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Filter and collect the precipitate, wash with five 10 mL volumes of water and dry at 105 ºC for 2 hours: it melts between 184 ºC and 187 ºC.

(3) A solution of Homatropine Hydrobromide (1 in 20) responds to the Qualitative Tests for bromide.

pH Dissolve 1.0 g of Homatropine Hydrobromide in 50 mL of water: the pH of this solution is between 5.7 and 7.0.

Purity (1) ***Acid*—**Dissolve 1.0 g of Homatropine Hydrobromide in 20 mL of water and add 0.40 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(2) ***Atropine, hyoscyamine and scopolamine*—** Take 10 mg of Homatropine Hydrobromide, add 5 drops of nitric acid, evaporate on a water-bath to dryness and cool. Dissolve the residue in 1 mL of *N,N*-dimethylformamide and add 5 to 6 drops of tetraethylammonium hydroxide TS: no red-purple color is produced.

(3) ***Other alkaloids*—**Dissolve 0.15 g of Homatropine Hydrobromide in 3 mL of water and use this solution as the test solution.

(i) Take 1 mL of the test solution, add 2 to 3 drops of tannic acid TS: no precipitate is produced.

(ii) Take 1 mL of the test solution, add 2 to 3 drops each of dilute hydrochloric acid and hexachloroplatinic (IV) acid TS: no precipitate is produced.

Loss on Drying Not more than 1.5 % (0.5 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.2 % (0.2 g).

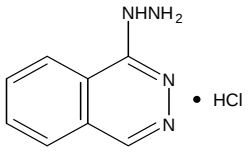
Assay Dissolve by warming about 0.4 g of Homatropine Hydrobromide in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3). Cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detdction Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.626 mg of $C_{16}H_{21}NO_3$.HBr

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Hydralazine Hydrochloride



$C_8H_8N_4$·HCl: 196.64

1-Hydrazinylphthalazine hydrochloride [*304-20-1*]

Hydralazine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of hydralazine hydrochloride ($C_8H_8N_4$·HCl).

Description Hydrazine Hydrochloride is a white, crystalline powder and is odorless and has a bitter taste. Hydrazine Hydrochloride is soluble in water, slightly soluble in ethanol (95) and practically insoluble in ether.

Melting point—About 275 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Hydralazine Hydrochloride and Hydralazine Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Hydralazine Hydrochloride and Hydralazine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Hydralazine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity (1) ***Clarity and color of solution*—**Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the solution is clear, and colorless to pale yellow.

(2) ***Heavy metals*—**Proceed with 1.0 g of Hydralazine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances*—**Weigh accurately 25 mg of Hydralazine Hydrochloride, add 30 mL of 0.1 mol/L acetic acid, sonicate to dissolve, cool, add 0.1 mol/L acetic acid to make 50 mL, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography, determine each peak area other than the solvent by the automatic integration method, and calculate the amount of each related substance by the area percentage method: the total amount of related substances is not more than 1.0 %.

Amount (%) of each related substance

$$= 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance obtained from the test solution

A_S: Total area of all peaks obtained from the test solution other than the solvent peak

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with cyanopropylsilyl silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetra *n*-butylammonium bromide in 770 mL of water, and add 230 mL of acetonitrile. Adjust the pH to 3.0 with 0.1 mol/L sulfuric acid.

Flow rate: About 1 mL/minute

System suitability

System performance: Dissolve 25 mg of Hydralazine Hydrochloride RS and 5 mg of phthalazine in 100 mL of 0.1 mol/L acetic acid. Pipet 5 mL of this solution, add 0.1 mol/L acetic acid to make 50 mL, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution under the above operating conditions, the relative retention time of phthalazine with respect to hydralazine hydrochloride is 0.65 with the resolution between these peaks being not less than 4.0.

(4) ***Hydrazine***—Weigh accurately 20 mg of Hydralazine Hydrochloride, dissolve in 1.0 mL of water, add 4 mL of benzaldehyde solution, and shake for 20 minutes using an appropriate apparatus. Pipet 2.0 mL of this solution, pass through a solid phase extraction column, and elute into a 5 mL volumetric flask. Wash the column with two 1.5 mL volumes of a mixture of water and acetonitrile (3 : 7), combine the washings with the eluate, add a mixture of water and acetonitrile (3 : 7) to make 5 mL, and use this solution as the test solution. Separately, weigh accurately about 65 mg of hydrazine dihydrochloride, and add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. To 1 mL of this solution add water to make 20 mL. Pipet 1.0 mL of this solution, add 4 mL of benzaldehyde solution, and shake for 20 minutes using an appropriate apparatus. Pipet 2.0 mL of this solution, add a mixture of water and acetonitrile (3 : 7) to make 5 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of each solution by the automatic integration method, and determine the peak areas, A_T and A_S, of hydrazine (not more than 10 ppm).

$$\text{Amount (ppm) of hydrazine} = 1000 \times \frac{32.05}{104.97} \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

32.05: Molecular weight of hydrazine

104.97: Molecular weight of hydrazine dihydrochloride

C_S: Concentration (μg/mL) of hydrazine dihydrochloride ($(NH_2)_2 \cdot 2HCl$) in the standard solution

C_T: Concentration (mg/mL) of hydralazine hydrochloride in the test solution

A_T: Peak area of hydrazine obtained from the test solution

A_S: Peak area of hydrazine obtained from the standard solution

Benzaldehyde solution—Pipet 1.0 mL of benzaldehyde, and dilute with a mixture of methanol and water (9 : 1) to make 100 mL.

Solid phase extraction column—Pack the column with benzenesulfonic acid strong cation-exchange packing with a sorbent-mass to column volume ratio of 0.5 g/3 mL or equivalent. Before use, wash the column with two 2.0 mL volumes of hexane, dry immediately in vacuum for 2 minutes, and wash with two 2.0 mL volumes each of methanol, water, and pH 7.0 phosphate buffer solution.

pH 7.0 Phosphate buffer solution—Dissolve 5.82 g of sodium hydrogen phosphate and 3.81 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 7.0 ± 0.1 with 1 mol/L sodium hydroxide solution or 1 mol/L phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 310 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 0.3 g of disodium ethylenediaminetetraacetate dihydrate in 300 mL of water, and add acetonitrile to make 1000 mL.

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative retention time of hydrazine derivative with respect to hydralazine derivative is about 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 8 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.15 g of Hydralazine Hydrochloride, previously dried, transfer to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, add 5 mL of chloroform and titrate with 0.05 mol/L potassium iodide VS while shaking until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color no more reappears in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Hydralazine Hydrochloride for Injection

Hydralazine Hydrochloride for Injection is a preparation for injection which is reconstituted before use. Hydralazine Hydrochloride contains not less than 99.0 % and not more than 113.0 % of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$: 196.64).

Method of Preparation Prepare as directed under Injections, with Hydralazine Hydrochloride.

Description Hydralazine Hydrochloride for Injection appears as white to pale yellow powder of mass, is odorless, and has a bitter taste.

Identification Determine the absorption spectrum of a solution of Hydralazine Hydrochloride for Injection (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm and between 313 nm and 317 nm.

pH Dissolve 1.0 g of Hydralazine Hydrochloride for Injection in 50 mL of water: the pH of this solution is between 3.5 and 4.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 5.0 EU/mg of hydralazine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Unit It meets the requirement when the Mass Variation test is performed.

Assay Weigh accurately the contents not less than 10 samples of Hydralazine Hydrochloride for Injection. Weigh accurately about 0.15 g of Hydralazine Hydrochloride, transfer to a stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, add 5 mL of chloroform and titrate with 0.05 mol/L potassium iodate VS while shaking until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color no more reappears in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4 \cdot HCl$

Containers and Storage ***Containers***—Hermetic containers.

Hydralazine Hydrochloride Tablets

Hydralazine Hydrochloride Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$: 196.64).

Method of Preparation Prepare as directed under Tablets, with Hydralazine Hydrochloride.

Identification Weigh a portion of powdered Hydralazine Hydrochloride Tablets, equivalent to 25 mg of Hydralazine Hydrochloride according to the labeled amount, add 100 mL of water, mix well and filter, if necessary. Take 2 mL of this solution, add water to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm and between 313 nm and 317 nm.

Dissolution Test Perform the test with 1 tablet of Hydralazine Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 30 mL or more of the dissolved solution after 45 minutes from the start of the Dissolution test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL, so that each mL of the filtrate contains about 11 μg of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 50 mg of Hydralazine Hydrochloride RS, previously dried at 105°C for 3 hours and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively at 260 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Hydralazine Hydrochloride Tablets in 45 minutes should be not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

W_S: Amount (mg) of Hydralazine Hydrochloride RS,
C: Labeled amount (mg) of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately not less than 20 Hydralazine Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$), transfer to a stoppered flask and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Hydrochloric Acid

HCl: 36.46

Chlorane [*7647-01-0*]

Hydrochloric Acid contains not less than 35.0 % and not more than 38.0 % of hydrogen chloride (HCl).

Description Hydrochloric Acid is colorless liquid having a pungent odor.
Hydrochloric Acid is fuming but ceases to fume when it is diluted with 2 volumes of water.

Specific gravity d_{20}^{20} —About 1.18.

Identification (1) Allow a glass stick wet with ammonia water to come near the surface of Hydrochloric Acid: a remarkable white smoke evolves.

(2) A solution of Hydrochloric Acid (1 in 100) changes blue litmus paper to red and responds to the Qualitative Tests for chloride.

Purity (1) ***Sulfate***—Take 15 mL of Hydrochloric Acid, add water to make 50 mL and use this solution as the test solution. To 3 mL of the test solution, add 5 mL of water and 5 drops of barium chloride TS and allow to stand for 1 hour: no turbidity is produced.

(2) ***Sulfite***—Take 3 mL of the test solution obtained in (1), add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) ***Bromide or iodide***—Place 10 mL of the test solution obtained in (1) in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS and shake well: the chloroform layer remains colorless.

(4) ***Bromine or chlorine***—Place 10 mL of the test solution obtained in (1) in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) ***Heavy metals***—Evaporate 5 mL of Hydrochloric Acid on a water-bath to dryness and add 2 mL of dilute acetic acid and water to the residue to make 50 mL. Perform the test using this solution the test solution. Prepare the control solution as follows: to 3.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(6) ***Mercury***—Dilute 20 mL of hydrochloric Acid with water to make exactly 100 mL and use the solution as the test solution. Perform the test with this test solution as directed under the Atomic Absorption Spectrophotometry (cold vapor type). Place the test solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of stannous chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air and determine the absorbance, A_T, of the test solution after the recorder reading has risen rapidly and becomes constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of standard mercury solution, add water to make exactly 100 mL and determine the absorbance, A_S, of the solution obtained by the same procedure as used for the test solution: A_T is smaller than A_S (not more than 0.04 ppm).

(7) ***Arsenic***—Prepare the test solution with 1.7 mL of Hydrochloric Acid according to Method 1 and perform the test (not more than 1 ppm).

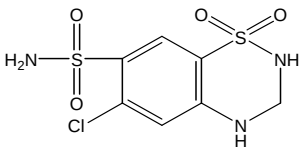
Residue on Ignition Pipet 10 mL of Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness and ignite: not more than 1.0 mg of residue remains.

Assay Weigh accurately a glass-stoppered flask containing 20 mL of water, add about 3 mL of Hydrochloric Acid and weigh accurately again. Dilute with 25 mL of water and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS
= 36.461 mg of HCl

Containers and Storage ***Containers***—Tight containers.

Hydrochlorothiazide



$C_7H_8ClN_3O_4S_2$: 297.74

6-Chloro-1,1-dioxo-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine-7-sulfonamide [*58-93-5*]

Hydrochlorothiazide contains not less than 99.0 % and not more than 101.0 % of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), calculated on the dried basis.

Description Hydrochlorothiazide appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste.
Hydrochlorothiazide is freely soluble in acetone, sparingly soluble in methanol, very slightly soluble in water or in ethanol (95) and practically insoluble in ether.
Hydrochlorothiazide dissolves in sodium hydroxide TS.
Melting point—About 267 ºC (with decomposition).

Identification (1) Take 5 mg of Hydrochlorothiazide, add 5 mL of chromotropic acid TS and allow to stand for 5 minutes: a purple color is observed.

(2) Fuse a mixture of 0.1 g of Hydrochlorothiazide and 0.5 g of sodium carbonate decahydrate cautiously: the gas evolved changes moistened red litmus paper to blue. After cooling, crush with a glass rod, add 10 mL of water, stir and filter. Take 4 mL of the filtrate, add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is produced,

(3) Take 4 mL of the filtrate obtained in (2), add 5 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white precipitate is produced.

(4) Dissolve 12 mg each of Hydrochlorothiazide and Hydrochlorothiazide RS in 100 mL of sodium hydroxide TS. To 10 mL each of these solutions, add water to make 100 mL and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Chloride***—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS, add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036 %).

(2) ***Sulfate***—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS, add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Hydrochlorothiazide according to Method 2 and perform the test. Prepare the test solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Primary aromatic amines***—Dissolve 80 mg of Hydrochlorothiazide in acetone to make exactly 100 mL. Measure exactly 1 mL of the solution, add 3.0 mL of dilute hydrochloric acid, 3.0 mL of water and 0.15 mL of sodium nitrite TS, shake and allow to stand for 1 minute. Shake this solution with 1.0 mL of ammonium sulfamate TS, allow to stand for 3 minutes, then add 1.0 mL of *N*-(l-naphthyl)-*N*-diethylethylenediamine oxalate TS, shake and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 1.0 mL of acetone in the same manner as the blank: the absorbance at 525 nm is not more than 0.10.

(5) ***Related substances***—Weigh accurately 32 mg of Hydrochlorothiazide, add 70 mL of the diluent, sonicate for 10 minutes to dissolve, cool to room temperature, add the diluent to make exactly 100 mL, filter through a filter with a pore size of 0.45 μm, and use as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances by the following equation: hydrochlorothiazide related substance I {4-amino-6-chloro-1,3-benzenedisulfonamide} is not more than 1.0 %, and any other related substance is not more than 0.5 %. The total amount of related substances other than hydrochlorothiazide related substance I is not more than 0.9 %. Use the peak areas of hydrochlorothiazide related substance I and chlorothiazide after dividing by their relative response factors, 0.54 and 0.63, respectively.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance obtained from the test solution
A_S: Peak area of all related substances obtained from the test solution

Diluent—A mixture of sodium phosphate solution and acetonitrile (7 : 3)

Sodium phosphate solution—Weigh accurately 2.76 g of sodium dihydrogen phosphate monohydrate, dissolve in 990 mL of water, adjust the pH to 2.7 with phosphoric acid, and add water to make 1000 mL.

Operating conditions
Proceed as directed in the operating conditions in the Assay.
System suitability
System performance: Dissolve a suitable amount each of Hydrochlorothiazide RS, Chlorothiazide RS, and hydrochlorothiazide related substance I, dissolve in the diluent to make a solution so that each mL contains 0.32 mg, 0.0032 mg, and 0.0032 mg, respectively, filter through a filter with a pore size of 0.45 μm, and use as the system suitability solution. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the resolution between

the peaks of hydrochlorothiazide related substance I and chlorothiazide is not less than 2.0, the resolution between the peaks of chlorothiazide and hydrochlorothiazide is not less than 1.5, and the symmetry factor of each peak is not more than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of hydrochlorothiazide related substance I and chlorothiazide is not more than 5.0 %. Weigh accurately a suitable amount of Hydrochlorothiazide RS, and dissolve in the diluent so that each mL contains 0.16 μg. When the test is repeated 6 times with 10 μL each of this solution under the above operating conditions, the relative standard deviation is not more than 25 %.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 32 mg each of Hydrochlorothiazide and Hydrochlorothiazide RS, add 70 mL of the diluent, sonicate for 10 minutes to dissolve, cool to room temperature, add the diluent to make exactly 100 mL, filter through a membrane filter with a pore size of 0.45 μm, and use these solutions as the test solution and standard solution, respectively. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S of hydrochlorothiazide in each solution.

$$\text{Amount (mg) of hydrochlorothiazide } (C_7H_8ClN_3O_4S_2) = \text{Amount (mg) of Hydrochlorothiazide RS} \times \frac{A_T}{A_S}$$

Diluent—A mixture of sodium phosphate solution and acetonitrile (7 : 3)

Sodium phosphate solution—Weigh accurately 2.76 g of sodium dihydrogen phosphate monohydrate, dissolve in 990 mL of water, adjust the pH to 2.7 with phosphoric acid, and add water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of acetonitrile and methanol (3 : 1)

Mobile phase B: A solution of anhydrous formic acid (5 in 1000)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-5	3	97
5-14	3→36	97→64
14-18	36→3	64→97
18-20	3	97

Flow rate: About 1.0 mL/minute

Column temperature: A constant temperature of about 35 °C

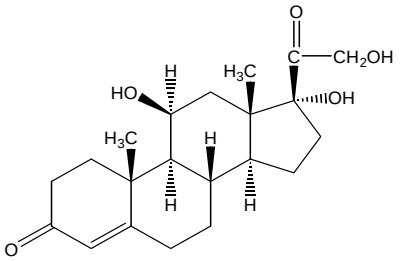
System suitability

System performance: Dissolve a suitable amount each of Hydrochlorothiazide RS, Chlorothiazide RS, and hydrochlorothiazide related substance I in the diluent to make a solution so that each mL contains 0.32 mg, 0.0032 mg, and 0.0032 mg, respectively, filter through a filter with a pore size of 0.45 μm, and use this solution as the system suitability solution. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the relative retention times of hydrochlorothiazide related substance I and chlorothiazide with respect to hydrochlorothiazide are about 0.5 and about 0.8, respectively. The resolution between the peaks of hydrochlorothiazide related substance I and chlorothiazide is not less than 2.0, the resolution between the peaks of chlorothiazide and hydrochlorothiazide is not less than 1.5, and the symmetry factor of each peak is not more than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Hydrocortisone



$C_{21}H_{30}O_5$: 362.47

(8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-

6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3(2*H*)-one [*50-23-7*]

Hydrocortisone, when dried, contains not less than 97.0 % and not more than 102.0 % of hydrocortisone ($C_{21}H_{30}O_5$).

Description Hydrocortisone appears as white crystalline powder and is odorless.
Hydrocortisone is sparingly soluble in methanol, in ethanol (95) and in 1,4-dioxane, slightly soluble in chloroform and very slightly soluble in ether and in water.

Melting point—212 ~ 220 ºC (with decomposition).

Identification (1) Take 2 mg of Hydrocortisone, add 2 mL of sulfuric acid: the solution shows a yellow-green fluorescence immediately and the color of the solution changes gradually from orange to dark red. Dilute carefully the solution with 10 mL of water: the color changes through yellow to orange-yellow with green fluorescence and a small amount of a flocculent precipitate is produced.

(2) Dissolve 10 mg of Hydrocortisone in 1 mL of methanol, add 1 mL of Fehling's TS and heat: a red precipitate is produced.

(3) Determine the infrared spectra of Hydrocortisone and Hydrocortisone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve the test and RS in ethanol (95), respectively, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +162 ~ +168° (after drying, 0.2 g, methanol, 25 mL, sonicate for 10 minutes, 100 mm).

Purity ***Related substances***—Dissolve 20 mg of Hydrocortisone in 10 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for Thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (17 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone and Hydrocortisone RS, previously dried and accurately weighed, in 20 mL each of a mixture of chloroform and methanol (9 : 1), add 10 mL each of the internal standard solution, then add a mixture of chloroform and methanol (9 : 1) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of hydrocortisone } (C_{21}H_{30}O_5) = \text{Amount (mg) of Hydrocortisone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of prednisone in a mixture of chloroform and methanol (9 : 1) (9 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20 ºC.

Mobile phase: A mixture of chloroform, methanol and acetic acid (100) (1000 : 20 : 1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone is about 15 minutes.

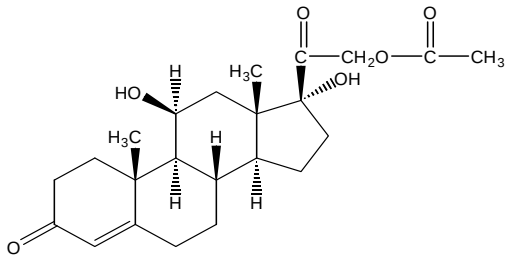
System suitability

System performance: When the procedure is run with 5 μL of the standard solution, as directed under the above operating conditions, the internal standard and hydrocortisone are eluted in this order with a resolution between their peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 1.0.

Containers and Storage ***Containers***—Tight containers.

Hydrocortisone Acetate



$C_{23}H_{32}O_6$: 404.50

2-((8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)-2-oxoethyl acetate [*50-03-3*]

Hydrocortisone Acetate, when dried, contains not less than 97.0 % and not more than 102.0 % of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Description Hydrocortisone Acetate appears as white crystals or crystalline powder and is odorless.
Hydrocortisone Acetate is sparingly soluble in 1,4-dioxane, slightly soluble in methanol, in ethanol (95) or in chloroform, very slightly soluble in ether and practically insoluble in water.

Melting point—About 220 °C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Acetate: the solution shows a yellowish green fluorescence immediately and the color of the solution gradually changes through orange to dark red. This solution shows a strong pale green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a pale green fluorescence and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 10 mg of Hydrocortisone Acetate in 1 mL of methanol by warming, add 1 mL of Fehling's TS and heat: an orange to red precipitate is formed.

(3) Take 50 mg of Hydrocortisone Acetate, add 2 mL of potassium hydroxide-ethanol TS and heat in a water-bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7) and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(4) Determine the infrared spectra of Hydrocortisone Acetate and Hydrocortisone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve the test sample and RS in ethanol (95), respectively, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: + 158 ~ + 165° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Purity ***Related substances***—Dissolve 0.040 g of Hydrocortisone Acetate in 25 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 2 mL of the test solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions as directed under the Thin-layer chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ether, methanol and water (160 : 30 : 8 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone Acetate and Hydrocortisone acetate RS, previously dried and accurately weighed, in methanol, add exactly 10 mL each of the internal standard solution, then add methanol to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone acetate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of hydrocortisone acetate } (C_{23}H_{32}O_6) = \text{Amount (mg) of Hydrocortisone Acetate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzyl *p*-hydroxybenzoate in methanol (1 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone Acetate is about 8 minutes.

System suitability

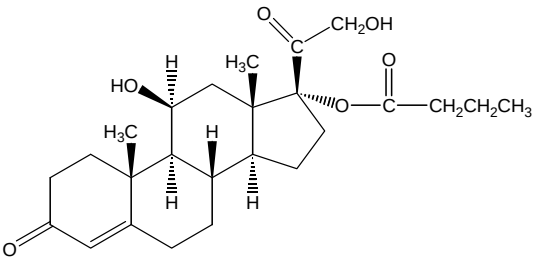
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone acetate and the

internal standard are eluted in this order with a resolution between their peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone acetate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Hydrocortisone Butyrate



$C_{25}H_{36}O_6$: 432.55

(8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11-hydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl butanoate [*13609-67-1*]

Hydrocortisone Butyrate, when dried, contains not less than 96.0 % and not more than 104.0 % of hydrocortisone butyrate ($C_{25}H_{36}O_6$).

Description Hydrocortisone Butyrate appears as white powder and is odorless.
Hydrocortisone Butyrate is freely soluble in tetrahydrofuran, in chloroform or in 1,2-dichlroethane, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in ether and practically insoluble in water.

Melting point—About 200 °C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Butyrate: the solution shows a yellowish green fluorescence immediately and the color of the solution gradually changes through orange to dark red. This solution shows a strong pale green fluorescence under ultraviolet light (main wavelength: 254 nm). Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a pale green fluorescence and a yellow-brown, flocculent precipitate is produced.

(2) Dissolve 10 mg of Hydrocortisone Butyrate in 1 mL of methanol by warming, add 1 mL of Fehling's TS and heat: an orange to red precipitate is formed.

(3) Take 50 mg of Hydrocortisone Butyrate, add 2 mL of potassium hydroxide-ethanol TS and heat on a water-bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7) and boil gently for 1 minute: the odor of ethyl butyrate is perceptible.

(4) Determine the infrared spectra of Hydrocortisone Butyrate and Hydrocortisone Butyrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers

Specific Optical Rotation $[\alpha]_D^{20}$: +48 ~ +52° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Hydrocortisone Butyrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 25 mg of Hydrocortisone Butyrate in 5 mL of tetrahydrofuran and use this solution as the test solution. Pipet 2 mL of this solution and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add tetrahydrofuran to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (470 : 30 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the test solution are not more than two in number and not more intense than those from the standard solution in color.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

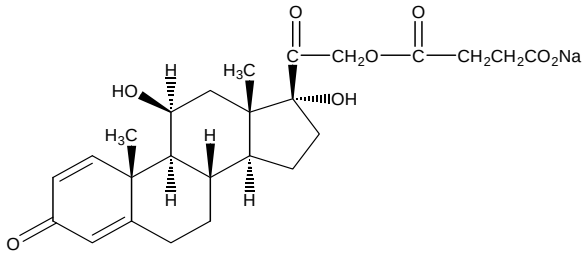
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance, *A,* of this solution at the wavelength of a maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of hydrocortisone butyrate } (C_{25}H_{36}O_6) = \frac{A}{375} \times 25000$$

Containers and Storage ***Containers***—Tight containers.

Hydrocortisone Sodium Succinate



$C_{25}H_{33}NaO_8$: 484.51

Sodium 4-[2-[(8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoate [*125-04-2*]

Hydrocortisone Sodium Succinate contains not less than 97.0 % and not more than 103.0 % of hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$), calculated on the dried basis.

Description Hydrocortisone Sodium Succinate appears as white powder or mass and is odorless.
Hydrocortisone Sodium Succinate is freely soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.
Hydrocortisone Sodium Succinate is hygroscopic.
Hydrocortisone Sodium Succinate is gradually colored by light.

Identification (1) Dissolve 0.2 g of Hydrocortisone Sodium Succinate in 20 mL of water. and add 0.5 mL of dilute hydrochloric acid with stirring: a white precipitate is formed. Filter and collect the precipitate. wash it with two 10-mL portions of water, and dry at 105 °C for 3 hours. To 3 mg of this dried matter add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong pale green fluorescence under ultraviolet light. Add carefully 10 ml of water to this solution: the color changes from yellow to orange with a pale green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Dissolve 10 mg of the dried matter obtained in (1) in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.1 g of the dried matter obtained in (1) add 2 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Filter the solution to remove the precipitate formed, mix the filtrate with 1 mL of dilute hydrochloric acid, filter if necessary, then adjust the solution to a pH of about 6 with diluted ammonia TS (1 in 10), and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Determine the infrared spectra of the dried matter obtained in (1) and Hydrocortisone Succinate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the specera, dissolve Hydrocortisone Sodium Succinate and Hydrocortisone Sodium Succinate RS in methanol, respectively, then evaporate the methanol to dryness, and repeal the test on the residues.

(5) Hydrocortisone Sodium Succinate responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +135 ~ +145° (0.1 g, calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Hydrocortisone Sodium Succinate in 10 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Dissolve 25 mg of Hydrocortisone Sodium Succinate in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 25 mg of hydrocortisone RS in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution (1). Pipet 6 mL of the standard solution (1), add methanol to make exactly 10 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under the Thin-layer chromatography. Spot 3 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150 : 10 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution corresponding to the spot from the standard solution (1) is not more intense than the spot from the standard solution (1). Any spot other than the principal spot and the above spot obtained from the test solution is not more than one and is not more intense than the spot from the standard solution (2).

Loss on Drying Not more than 2.0 % (0.5 g, 105 ºC, 3 hours).

Assay Weigh accurately about 10 mg of Hydrocortisone Sodium Succinate and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Hydrocortisone Succinate RS, previously dried at 105 ºC for 3 hours, proceed in the same manner as directed for the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 240 nm as directed under Ultraviolet-

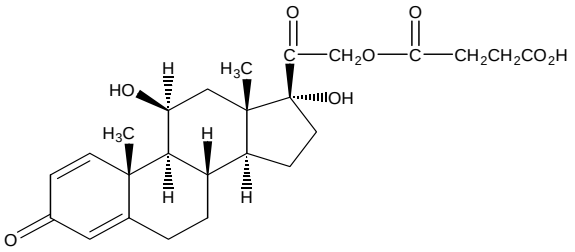
visible Spectrophotometry.

$$\text{Amount (mg) of hydrocortisone sodium succinate } (C_{25}H_{33}NaO_8) = \text{Amount (mg) of Hydrocortisone Sodium Succinate RS} \times \frac{A_T}{A_S} \times 1.0475$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Hydrocortisone Succinate



$C_{25}H_{34}O_8$: 462.53

4-[2-[(8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoic acid [*2203-97-6*]

Hydrocortisone Succinate, when dried, contains not less than 97.0 % and not more than 103.0 % of hydrocortisone succinate ($C_{25}H_{34}O_8$).

Description Hydrocortisone Succinate appears as white crystalline powder and is odorless.

Hydrocortisone Succinate is very soluble in methanol, freely soluble in ethanol (99.5), sparingly soluble in ethanol (95), very slightly soluble in ether and practically insoluble in water.

Identification (1) Take 3 mg of Hydrocortisone Succinate, add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately and the color of the solution gradually changes through orange to dark red. This solution shows a strong pale green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange with a pale green fluorescence and a yellow-brown flocculent precipitate is produced.

(2) Determine the infrared spectra of Hydrocortisone Succinate and Hydrocortisone Succinate RS, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve Hydrocortisone Succinate and Hydrocortisone Succinate RS in methanol, respectively, evaporate to dryness and perform the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +147 ~ +153° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Hydrocortisone Succinate in 10 mL of methanol: the solution is clear and colorless.

(2) ***Related substances***—Dissolve 25 mg of Hydrocortisone Succinate in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 25 mg of Hydrocortisone RS in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 3 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150 : 10 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 2.0 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 50 mg each of Hydrocortisone Succinate and Hydrocortisone Succinate RS, previously dried and dissolve in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone succinate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of hydrocortisone succinate } (C_{25}H_{34}O_8) = \text{Amount (mg) of Hydrocortisone Succinate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxy benzoate in methanol (1 in 2500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of

about 25 °C.

Mobile phase: A mixture of acetic acid-sodium acetate buffer solution, pH 4.0 and acetonitrile (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone succinate is about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, hydrocortisone succinate and the internal standard in this order with the resolution between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution as directed under the above operating conditions, the relative deviation of the peak area of hydrocortisone succinate is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Hydrocortisone Tablets

Hydrocortisone Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$: 362.46).

Method of Preparation Prepare as directed under Tablets, with Hydrocortisone.

Identification Weigh a portion of powdered Hydrocortisone Tablets, equivalent to about 50 mg of Hydrocortisone according to the labeled amount, add 15 mL of hexane, shake for 15 minutes and remove the hexane. Take the residue, add 10 mL of hexane again, shake for 15 minutes, remove the hexane, add 10 mL of peroxide-free ether, shake for 15 minutes, remove the ether, add 25 mL of ethanol (99.5), shake for 15 minutes, filter and evaporate the filtrate in a water-bath to dryness. Proceed with the residue as directed in the Identification (3) under Hydrocortisone.

Dissolution Test Perform the test with 1 tablet of Hydrocortisone Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 30 minutes from starting of the test, filter, dilute with the dissolution solution, if necessary and use this solution as the test solution. Separately, weigh accurately a portion of Hydrocortisone RS, previously dried at 105 ºC for 3 hours, add the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry using the test solution as the blank.

The dissolution rate of Hydrocortisone Tablets in 30 minutes is not less than 70 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

Transfer 1 tablet of Hydrocortisone Tablets to a suitable container and add about 0.3 mL of water directly on the tablet. Allow the tablet to stand for about 5 minutes. Shake the container to break up the tablet and sonicate to ensure complete disintegration. Add 4 to 5 small glass beads and 50.0 mL of the internal standard solution to the container. Shake the container for about 30 minutes. Dilute a V mL of the clear supernatant liquid, accurately measured, with a known, accurately measured volume of the internal standard solution to obtain a solution having a known concentration of 0.1 mg per mL and use this solution as the test solution. Separately, weigh accurately a portion of Hydrocortisone RS, previously dried at 105 ºC for 3 hours, dissolve in the internal standard solution to obtain a solution having a known concentration of 0.1 mg per mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Hydrocortisone to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of hydrocortisone } (C_{21}H_{30}O_5) = 50 \times \frac{V'}{V} \times C \times \frac{Q_T}{Q_S}$$

V': Final volume (mL) of the test solution,
C: Concentration (mg/mL) of standard solution.

Internal standard solution—A solution of prednisone in a saturated chloroform in water (6 in 100000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, having porous silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of butyl chloride, water-saturated butyl chloride, methanol, acetic acid (100), and tetrahydrofuran (95 : 95 : 7 : 6 : 4).

System suitability

System performance: When the procedure is run with 10 μL of the standard solution, as directed under the above operating conditions, the resolution between the peaks of hydrocortisone and the internal standard is not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution as directed under the above conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 2.0 %.

Assay Weigh accurately and powder not less than 20 Hydrocortisone Tablets, weigh accurately a portion of the powder, equivalent to about 5 mg of hydrocortisone ($C_{21}H_{30}O_5$), transfer to a centrifuge and add 50 mL of the internal standard solution. Shake vigorously for 30 minutes, centrifuge and use the clear supernatant liquid as the test solution. Proceed as directed in the Uniformity of Dosage Units under Hydrocortisone

$$\text{Amount (mg) of hydrocortisone } (C_{21}H_{30}O_5) = 50 \times C \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of the standard solution

Containers and Storage ***Containers***—Well-closed containers.

Hydrogen Peroxide Solution

[*7722-84-1*]

Hydrogen Peroxide Solution contains not less than 2.5 % and not more than 3.5 % (w/v) of hydrogen peroxide (H_2O_2: 34.02).
Hydrogen Peroxide Solution contains not more than 0.5 w/v % of suitable preservatives.

Description Hydrogen Peroxide Solution is a colorless, clear liquid and is odorless or has ozone-like odor.
Hydrogen Peroxide Solution is acidic and produce bubbles in the opening of the container.
Hydrogen Peroxide Solution's decomposition is accelerated by contacting oxidants or reductants.
Hydrogen Peroxide Solution can be degraded by heat.
Hydrogen Peroxide Solution needs to be protected from light.

Specific gravity d_{20}^{20} —About 1.01.

Identification Shake 1 mL of Hydrogen Peroxide Solution with 10 mL of water containing 1 drop of dilute sulfuric acid, and add 2 mL of ether. Subsequently, 1 drop of potassium dichromate TS is added to produce a pale blue color in water layer which, upon agitation and standing, passes into the ether layer.

Purity (1) ***Acidity***—Take 25.0 mL of Hydrogen Peroxide Solution, add 2 drops of phenolphthalein and 2.5 mL of 0.1 mol/L sodium hydroxide: a red color develops.

(2) ***Heavy metals***—Take 5 mL of Hydrogen Peroxide Solution, add 20 mL of water and 2 mL of ammonia TS and evaporate on a water-bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid by heating and add water to make 50 mL. Perform the test with this solution as the test solution. Pipet 2.5 mL of standard lead solution and add 2 mL of dilute acetic acid and water to make 50 mL and use this solution as the reference solution (not more than 5 ppm).

(3) ***Arsenic***—To 1.0 mL of Hydrogen Peroxide Solution, add 1 mL of ammonia TS and evaporate on a water-bath to dryness. Proceed with the residue according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Barium***—To 1.0 mL add two drops of 2 N sulfuric acid: no turbidity or precipitate is produced within 10 minutes.

(5) ***Organic preservatives***—Pipet 100.0 mL of Hydrogen Peroxide Solution and extract sequentially with 50 mL, 25 mL and 25 mL volumes of a mixture of chloroform and ether (3 : 2), place the combined extracts in the container, previously weighed, evaporate at room temperature and dry the residue in a desiccator with silica gel for 2 hours: not more than 50 mg.

(6) ***Non volatile residue***—Pipet 2.0 mL of Hydrogen Peroxide Solution, evaporate on a water-bath to dryness and dry residue at 105 ºC for 1 hour: not more than 20 mg.

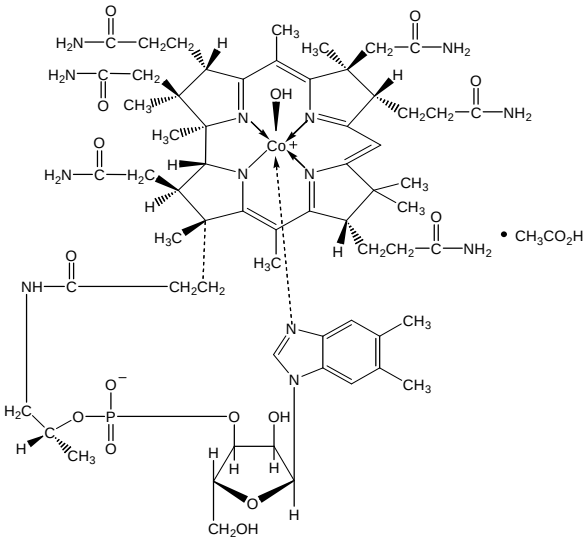
Assay Place accurately about 2 mL of Hydrogen Peroxide Solution to a flask containing 20 mL of water, add 20 mL of dilute sulfuric acid and titrate with 0.02 mol/L potassium permanganate VS.

$$\text{Each mL of 0.02 mol/L potassium permanganate VS} = 1.701 \text{ mg of } H_2O_2$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 30 ºC.

Hydroxocobalamin Acetate



$C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2$: 1406.41

Cobaltous [(2*R*,3*S*,4*R*,5*S*)-5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-

(hydroxymethyl)tetrahydrofuran-3-yl] [(1*R*)-1-methyl-2-[3-[(2*R*,3*R*,4*Z*,7*S*,9*Z*,12*S*,13*S*,14*Z*,17*S*,18*S*,19*R*)-2,13,18-tris(2-amino-2-oxo-ethyl)-7,12,17-tris(3-amino-3-oxo-propyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1*H*-corrin-21-id-3-yl]propanoylamino]ethyl] phosphate hydrate [*22465-48-1*]

Hydroxocobalamin Acetate contains not less than 95.0 % and not more than 101.0 % of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2$), calculated on the dried basis.

Description Hydroxocobalamin Acetate appears as deep red crystals or powder and is odorless.
Hydroxocobalamin Acetate is freely soluble in water, slightly soluble in ethanol (95) and practically insoluble in ether.
Hydroxocobalamin Acetate is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Hydroxocobalamin Acetate and Hydroxocobalamin Acetate RS in acetic acid-sodium acetate buffer solution, pH 4.5, (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Hydroxocobalamin Acetate with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until the solution devellops a pale red. Then add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium l-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange color develops immediately. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Add 0.5 mL of ethanol (99.5) and 1 mL of sulfuric acid to 20 mg of Hydroxocobalamin Acetate and heat the mixture: the odor of ethyl acetate is perceptible.

Purity ***Cyanocobalamin and colored related substances***—Dissolve 50 mg of Hydroxocobalamin Acetate in exactly 5 mL each of acetic acid-sodium acetate buffer solution, pH 5.0, in two tubes. Take one tube, add 0.15 mL of potassium thiocyanate TS, allow to stand for 30 minutes and use this solution as the test solution (1). To the other tube, add 0.10 mL of potassium cyanide TS, allow to stand for 30 minutes and use this solution as the test solution (2). Separately, dissolve 3.0 mg of Cyanocobalamin RS in exactly 10 mL of acetic acid-sodium acetate buffer solution, pH 5.0 and use this solution as the standard solution. Perform the test with the test solutions (1) and (2) and the standard solution as directed under the Thin-layer Chromatography. Apply 20 μL each of the test solutions (1) and (2) and the standard solution, 25 mm in length along the starting line, 10 mm apart from each other, on a plate of silica gel for thin-layer chromatography. Develop the plate for 18 hours with 2-butanol saturated with water, while supporting the plate at an angle of about 15° to a horizontal plane and air-dry the plate: the spot from the test solution (1) corresponding to that from the standard solution is not more intense than the spot from the standard solution and the spots other than the principal spot from the test solution (2) are not more intense than the spot from the standard solution.

Loss on Drying Not more than 12 % (0.05 g, in vacuum, at a pressure not exceeding 0.67 kPa, P_2O_5, l00 °C, 6 hours).

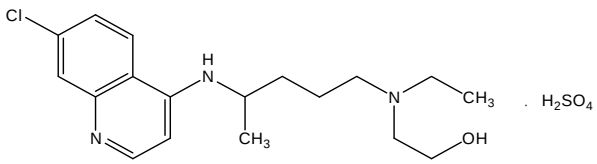
Assay Weigh accurately about 20 mg of Hydroxocobalamin Acetate and dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 2 mL of this solution into a 50-mL volumetric flask, add 1 mL of a solution of potassium cyanide (1 in 1000) and allow to stand for 30 minutes at ordinary temperature. Add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Cyanocobalamin RS after determining the loss on drying in the same manner as for Cyanocobalamin and dissolve in water to make exactly 50 mL. To 2 mL of this solution, exactly measured, add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 361 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of hydroxocobalamin acetate } (C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2) = \text{Amount (mg) of cyanocobalamin RS, calculated on the dried basis} \times \frac{A_T}{A_S} \times 1.0377$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Hydroxychloroquine Sulfate



$C_{18}H_{26}ClN_3O \cdot H_2SO_4$: 433.95

2-[4-[(7-Chloroquinolin-4-yl)amino]pentylethylamino]ethanol;sulfuric acid [*747-36-4*]

Hydroxychloroquine Sulfate contains not less than 98.0 % and not more than 102.0 % of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$), calculated on the dried basis.

Description Hydroxychloroquine Sulfate appears as white crystalline powder, is odorless, and has a bitter taste.
Hydroxychloroquine Sulfate is freely soluble in water, and practically insoluble in ethanol (95), in chloroform, and in ether.
Hydroxychloroquine Sulfate shows polymorphism.
The ordinary form of Hydroxychloroquine Sulfate melts at about 240 °C, while other forms melt at about 198 °C.

Identification (1) Determine the absorption spectra of solutions of Hydroxychloroquine Sulfate and Hydroxychloroquine Sulfate RS in diluted hydrochloric acid (1 in 100) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Hydroxychloroquine Sulfate and Hydroxychloroquine Sulfate RS, previously dried, as directed in the potassium bromide disk method under lnfrared Spectrophotornetry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Hydroxychloroquine Sulfate (1 100) responds to the Qualitative Tests for sulfate.

Purity ***Related substances***—Dissolve 0.10 g of Hydroxychloroquine Sulfate in a mixture of methanol and water (90 : 10) to make exactly 10 mL, and use this solution as the test solution. Separately, weigh exactly a portion of Hydroxychloroquine Sulfate RS, dissolve in a mixture of methanol and water (90 : 10) to make solutions containing 0.01, 0.05, 0.1, and 0.2 mg per mL, respectively, and use these solutions as the standard solutions. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), water and ammonia solution (28) (80 : 16 : 4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelengths: 254 nm and 366 nm): the spots other than the principal spot from the test solution are not more intense than the spots from the standard solution.

Loss on Drying Not more than 2.0 % (105 °C, 2 hours).

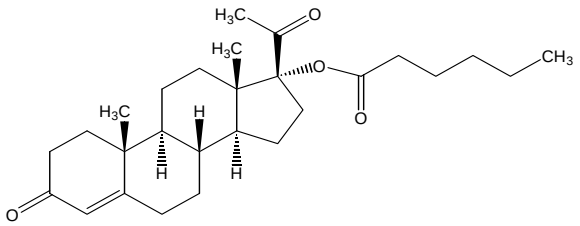
Assay Weigh accurately about 0.1 g each of Hydroxychloroquine Sulfate and Hydroxychloroquine Sulfate RS, dissolve in 5 mL of water, and add diluted hydrochloric acid (1 in 100) to make exactly 100 mL. Pipet 1.0 mL each of these solutions, add diluted hydrochloric acid (1 in 100) to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorbance near 343 nm.

$$\text{Amount (mg) of hydroxychloroquine sulfate } (C_{18}H_{26}ClN_3O \cdot H_2SO_4) = \text{Amount (mg) of Hydroxychloroquine Sulfate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Hydroxyprogesterone Caproate



$C_{27}H_{40}O_4$: 428.60

(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-acetyl-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl hexanoate [*630-56-8*]

Hydroxyprogesterone Caproate, contains not less than 97.0 % and not more than 103.0 % of hydroxylprogesterone caproate ($C_{27}H_{40}O_4$), calculated on the anhydrous basis.

Description Hydroxyprogesterone Caproate appears as white to milky white crystalline powder and is odorless or has a slight odor.
Hydroxyprogesterone Caproate is soluble in ether, and practically insoluble in water.

Identification Determine the infrared spectra of Hydroxyprogesterone Caproate and Hydroxyprogesterone Caproate RS, previously dried in vacuum in a dessicator (silica gel) for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: + 58 ~ + 64° (0.1 g, calculated on the anhydrous basis, chloroform, 10 mL, 100 mm).

Melting Point 120 ~ 124 °C.

Purity (1) ***Free n-caproic acid***—To 0.20 g of Hydroxyprogesterone Caproate add 2 to 3 drops of phenolphthalein TS, dissolve in 25 mL of neutralized ethanol until a pale red color develops, and titrate with 0.02 mol/L sodium hydroxide VS: not more than 0.50 mL is consumed (not more than 0.58 %).

(2) ***Related substances***—Dissolve 0.1 g of Hydroxyprogesterone Caproate in 10 mL of chloroform and use this solution is the test solution. Separately, weigh accurately about 10 mg of Hydroxyprogesterone Caproate RS, previously dried in vacuum in a dessicator (silica gel) for 4 hours, and dissolve in chloroform to make 10 mL. Pipet 0.1 mL, 0.5 mL, 1.0 mL and 2.0 mL of this solution, add 10 mL of acetone exactly and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with the test solution and the standard solutions (1), (2), (3) and (4), as directed under thin-layer chromatography. Spot 20 μL each of the test solution and the standard solutions (1), (2), (3) and (4) on silica gel plate for the thin-layer chromatography. Develop the plates with a mixture chloroform and ethyl acetate (3:1) to a distance about 15 cm and air-dry the plate. Spray a mixture of sulfuric acid and ethanol (95) (1 : 9), prepared by adding sulfuric acid slowly and carefully to ethonal on an ice-bath, on the plates thoroughly and heat the plates until it charr. Using ultraviolet light (main wavelength 366 nm), the relative intensities of the spots from the test solution, except the principal spot, compared to that of each standard solution is not more than 2.0 %.

Water Not more than 0.1 % (5 g, volumetric titration, direct titration).

Assay Weigh accurately about 50 mg each of Hydroxyprogesterone Caproate and Hydroxyprogesterone Caproate RS, previously dried in vacuum in a dessicator (silica gel) for 4 hours, dissolve in ethanol (95) to make exactly 100 mL and mix by shaking. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry using ethanol (95) as the blank. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorbance near 240 nm.

$$\text{Amount (mg) of hydroxyprogesterone caproate } (C_{27}H_{40}O_4) = \text{Amount (mg) of Hydroxyprogesterone Caproate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Hydroxyprogesterone Caproate Injection

Hydroxyprogesterone Caproate Injection is an oily injection. Hydroxyprogesterone Caproate Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$: 428.60).

Method of Preparation Prepare as directed under Injections, with Hydroxyprogesterone Caproate.

Description Hydroxyprogesterone Caproate Injection appears as clear, colorless to pale yellow oily liquid.

Identification (1) Transfer a volume of Hydroxyprogesterone Caproate Injection, equivalent to 0.125 g of Hydroxyprogesterone Caproate according to the labeled amount, to a separatory funnel containing 10 mL of hexane, 8 mL of methanol and 2 mL of water and shake for 2 minutes and stand to separate. Titrate 3 mL of lower layer with sulfuric acid until the color of solution appears. Add 3 mL of methanol: a purple color appears. Light with long wavelength ultraviolet line shows a pale yellow fluorescence.

(2) Evaporate 4 mL of the test solution of the Assay to dryness by heating in a water-bath. Dissolve the residue in 0.5 mL of chloroform and use this solution as the test solution. Dissolve a suitable amount of Hydroxyprogesterone Caproate RS in a suitable amount of chloroform so as to contain 400 μg of hydroxyprogesterone caproate per mL. Use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform and ethyl acetate (3 : 1) at a distance about 10 cm and air-dry. Spray a mixture of sulfuric acid and ethanol (95) (1 : 3) on these plates thoroughly and heat the plates at 105 °C for 5 minutes: the R_f value of the principal yellow-green spot obtained from the test solution corresponds to that from the standard solution.

Water Not more than 0.2 % (5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

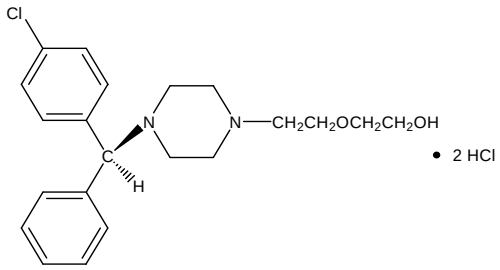
Assay Pipet a volume of Hydroxyprogesterone Caproate Injection, equivalent to 0.25 g of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$) according to the labeled amount, add methanol to make 250 mL and mix. Pipet 5 mL of this solution, add methanol to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Hydroxyprogesterone Caproate RS, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Pipet 5 mL each of the test solution and the standard solution to a stopered flask and add 10.0 mL of the isoniazid solution. Mix and allow to stand at 30 °C on a water-bath for about 45 minutes. Determine the absorbances of these solutions as directed under Ultraviolet-visible Spectrophotometry, A_T and A_S, at the wavelength of maximum absorbance near 380 nm, using a mixture of methanol and the isoniazid solution (1 : 2) as a blank.

$$\text{Amount (mg) of hydroxyprogesterone caproate } (C_{27}H_{40}O_4) = \text{Amount (mg) of Hydroxyprogesterone Caproate RS} \times \frac{A_T}{A_S} \times 5$$

Isoniazid solution—Dissolve 0.375 g of isoniazid in 0.47 mL of hydrochloric acid and 500 mL of methanol.

Containers and Storage ***Containers***—Hermetic containers.

Hydroxyzine Hydrochloride



and enantiomer

$C_{21}H_{27}ClN_2O_2 \cdot 2HCl$: 447.83

2-[2-[4-[(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy]ethanoldihydrochloride [*2192-20-3*]

Hydroxyzine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

Description Hydroxyzine Hydrochloride appears as white crystalline powder, is odorless and has a bitter taste.

Hydroxyzine Hydrochloride is very soluble in water, freely soluble in ethanol (95), in methanol or in acetic acid (100), very slightly soluble in acetic anhydride and practically insoluble in ether.

Melting point—About 200 °C (with decomposition).

Identification (1) Take 5 mL of a solution of Hydroxyzine Hydrochloride (1 in 100), add 2 to 3 drops of ammonium thiocyanate-cobaltous nitrate TS: a blue precipitate is produced.

(2) Determine the absorption spectra of solutions of Hydroxyzine Hydrochloride and Hydroxyzine Hydrochloride RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Hydroxyzine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Hydroxyzine Hydrochloride in 20 mL of water: the pH of this solution is between 1.3 and 2.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Hydroxyzine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Hydroxyzine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.20 g of Hydroxyzine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia solution (28) (150 : 95 : 1) to a distance of about 10 cm and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 3.0 % (1 g, 105 °C, 2 hours).

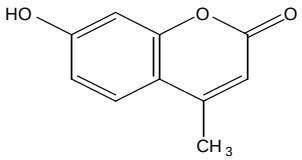
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g of Hydroxyzine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 22.392 mg of $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Hymecromone



$C_{10}H_8O_3$: 176.17

7-Hydroxy-4-methyl-2H-chromen-2-one [*90-33-5*]

Hymecromone, when dried, contains not less than 98.0 % and not more than 101.0 % of hymecromone ($C_{10}H_8O_3$).

Description Hymecromone appears as white crystals or crystalline powder, is odorless and tasteless.
Hymecromone is freely soluble in *N,N*-dimethylformamide, sparingly soluble in ethanol (95), in ethanol (99.5) or in acetone, slightly soluble in ether and practically insoluble in water.

Identification (1) Dissolve 2 mg of Hymecromone in 5 mL of ammonia-ammonium chloride buffer solution, pH 11.0: the solution shows an intense blue-purple fluorescence.

(2) Dissolve 25 mg of Hymecromone in 5 mL of diluted ethanol (1 in 2) and add 1 drop of iron (III) chloride TS: initially a blackish brown color is observed and when allowed to stand the color changes to yellow-brown.

(3) Determine the absorption spectra of solutions of Hymecromone and hymecromone RS in ethanol (99.5) (1 in 250000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Hymecromone and Hymecromone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 187 ~ 191 ºC.

Purity (1) ***Chloride***—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2 : 1) and add 6 mL of dilute nitric acid and a mixture of acetone and water (2 : 1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and a mixture of acetone and water (2 : 1) to make 50 mL (not more than 0.011 %).

(2) ***Sulfate***—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2 : 1) and add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2 : 1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS, add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Hymecromone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***— Prepare the test solution with 1.0 g of Hymecromone according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 80 mg of Hymecromone in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution and add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (10 : 1) to a distance of about 10 cm and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

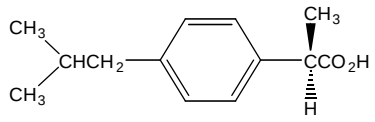
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Hymecromone, previously dried, dissolve in 90 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Separately, perform a blank determination with a solution prepared by adding 14 mL of water to 90 mL of *N,N*-dimethyl-formamide and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium
hydroxide VS = 17.617 mg of $C_{10}H_8O_3$

Containers and Storage ***Containers***—Tight containers.

Ibuprofen



and enantiomer

$C_{13}H_{18}O_2$: 206.28

2-[4-(2-Methylpropyl)phenyl]propanoic acid
[*15687-27-1*]

Ibuprofen, when dried, contains not less than 98.5 % and not more than 101.0 % of ibuprofen ($C_{13}H_{18}O_2$).

Description Ibuprofen appears as white crystalline powder.
Ibuprofen is very soluble in ethanol (95), in dehydrated ethanol, in acetone and sparingly soluble in water.
Ibuprofen dissolves in sodium hydroxide.

Identification (1) Determine the absorption specta of solutions of Ibuprofen and Ibuprofen RS in diluted sodium hydroxide TS (15 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavelengths.

(2) Determine the infrared specta of Ibuprofen and Ibuprofen RS, previously dried, as detected in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 75 ~ 77 °C.

Purity (1) ***Heavy metals***—Proceed with 3.0 g of Ibuprofen according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Ibuprofen according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.50 g of Ibuprofen in 5 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15 : 5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, not more than 0.67 kPa, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Ibuprofen, previously dried, dissolve in 50 mL of ethanol (95) and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.628 mg of $C_{13}H_{18}O_2$

Containers and Storage ***Containers***—Well-closed containers.

Ichthammol

[*8029-68-3*]

Ichthammol, calculated on the dried basis, contains not less than 2.5 % of ammonia NH_3: 17.030), not more not more than 8.0 % of ammonium sulfate [$(NH_4)_2SO_4$: 132.14] and not less than 10.0 % of total sulfur (as S: 32.07).

Description Ichthammol is a red-brown to blackish brown, viscous fluid and has a characteristic odor.
Ichthammol is miscible with water and is partially soluble in ethanol (95) or in ether.

Identification (1) Take 4 mL of a solution of Ichthammol (3 in 10), add 8 mL of hydrochloric acid: a yellow-brown to blackish brown, oily or resinous mass is produced. Cool the mass with ice to solidify and discard the water layer. Wash the residue with ether: a part of the mass dissolves but it does not dissolve completely even when it is washed until almost no color develops in the washing. Perform the following tests with this residue.

(i) Take 0.1 g of the residue, add 1 mL of a mixture of ether and ethanol (95) (1 : 1): it dissolves.

(ii) Take 0.1 g of the residue, add 2 mL of water: it dissolves. To 1 mL of this solution, add 0.4 mL of hydrochloric acid: a yellow-brown to blackish brown oily or resinous substance is produced.

(iii) Take 1 mL of the solution obtained in (ii), add 0.3 g of sodium chloride: a yellow-brown or blackish brown oily or resinous substance is produced.

(2) Boil 2 mL of a solution of Ichthammol (1 in 10) with 2 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

Loss on Drying Not more than 50 % (0.5 g, 105 ºC, 6 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Assay (1) ***Ammonia***—Weigh accurately about 5 g of Ichthammol, transfer to a Kjeldahl flask and add 60 mL of water, 1 mL of 1-octanol and 4.5 mL of a solution of sodium hydroxide (2 in 5). Connect the flask to a distilling tube with a spray trap and a condenser and immerse the lower outlet of the condenser in the receiver containing exactly 30 mL of 0.25 mol/L sulfuric acid VS. Distil slowly, collect about 50 mL of the distillate and titrate the excess sulfuric acid with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination and make any necessary correction.

Each mL of 0.25 mol/L sulfuric acid VS
= 8.515 mg of NH_3

(2) ***Ammonium sulfate***—Weigh accurately about 1 g of Ichthammol, add 25 mL of ethanol (95), stir thoroughly and filter. Wash with a mixture of ether and ethanol (95) (1:1) until the washings are clear and colorless. Dry the filter paper and the residue in air, dissolve the residue in 200 mL of hot water acidified slightly with hydrochloric aicd and filter. Boil the filtrate, add 30 mL of barium chloride TS slowly, heat for 30 minutes on a water bath and filter. Wash the precipitate with water, dry and ignite to constant weight. Weigh the residue as barium sulfate ($BaSO_4$: 233.39).

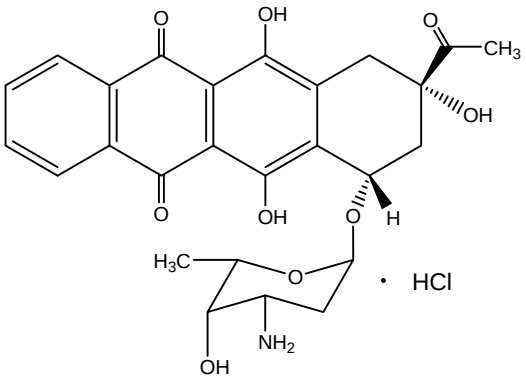
Amount (mg) of ammonium sulfate $[(NH_4)_2SO_4]$
= Amount (mg) of barium sulfate ($BaSO_4$) × 0.5662

(3) ***Total sulfur***—Weigh accurately about 0.6 g of Ichthammol, transfer to a 200-mL Kjeldahl flask and add 30 mL of water and 5 g of potassium chlorate, then add slowly 30 mL of nitric acid and evaporate the mixture to about 5 mL. Transfer the residue to a 300-mL beaker with the aid of 25 mL of hydrochloric acid and evaporate again to 5 mL. Add 100 mL of water, boil, filter and wash with water. Heat the combined filtrate and washings to boil, add gradually 30 mL of barium chloride TS, heat the mixture on a water-bath for 30 minutes and filter. Wash the precipitate with water, dry and ignite to constant weight. Weigh the residue as barium sulfate ($BaSO_4$).

Amount (mg) of total sulfur (S)
= Amount (mg) of barium sulfate ($BaSO_4$) × 0.13739

Containers and Storage ***Containers***—Tight containers.

Idarubicin Hydrochloride



$C_{26}H_{27}NO_9 \cdot HCl$: 533.95

(7*S*,9*S*)-9-Acetyl-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7*H*-tetracene-5,12-dione hydrochloride [*57852-57-0*]

Idarubicin Hydrochloride contains not less than 960 μg (potency) and not more than 1030 μg (potency) per mg of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$: 533.95), calculated on the anhydrous basis.

Description Idarubicin Hydrochloride appears as yellow-red powder.
Idarubicin Hydrochloride is sparingly soluble in methanol, slightly soluble in water or in ethanol (95), and practically insoluble in acetonitrile or in ether.

Identification (1) Determine the absorption spectra of solutions of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 2 mg of Idarubicin Hydrochloride in 3 mL of water, and add 1 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white turbidity is produced.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +191 ~ +197° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g (potency) of Idarubicin Hydrochloride in 20 mL of water is between 5.0 and 6.5.

Absorbance $E_{1\,cm}^{1\%}$ (482 nm): 204 ~ 210 (20 mg calculated on the anhydrous basis, methanol, 1000 mL).

Purity ***Related substances***—Proceed as directed in the assay, and calculate the ratio of the peak area of each related substance to the total peak area of the test solution excluding the solvent peak: the total amount of related substances is not more than 3.0 %, and each related substance is not more than 1.0 % with respect to the total amount.

Water Not more than 5.0 % (0.1 g, coulometric titration).

Sterility Test It meets the requirement, when Idarubicin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 8.9 EU/mg (potency) of idarubicin hydrochloride, when Idarubicin Hydrochloride is used in a sterile preparation.

Assay Weigh accurately about 10 mg (potency) each of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS, dissolve each in the mobile phase containing no sodium lauryl sulfate to make exactly 50 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of idarubicin in each solution.

$$\text{Amount [μg (potency)] of idarubicin hydrochloride } (C_{26}H_{27}NO_9\cdot HCl) = \text{Amount [μg (potency)] of Idarubicin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: Dissolve 10.2 g of potassium dihydrogen phosphate in water, and add 1 mL of phosphoric acid and water to make 750 mL. To this solution add 250 mL of tetrahydrofuran. To 500 mL of this solution add 0.72 g of sodium lauryl sulfate and 0.5 mL of *N,N*-dimethyl-*n*-octylamine, and adjust the pH to 4 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of idarubicin is about 15 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of idarubicin is not less than 3000.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of idarubicin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Idarubicin hydrochloride for Injection

Idarubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.
Idarubicin Hydrochloride for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of idarubicin hydrochloride ($C_{26}H_{27}NO_9\cdot HCl$: 533.95).

Method of Preparation Prepare as directed under Injections, with Idarubicin Hydrochloride.

Description Idarubicin Hydrochloride for Injection appears as yellow-red masses.

Identification (1) Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 2 mg (potency) of idarubicin hydrochloride according to the labeled amount, in 5 mL of sodium hydroxide TS: a blue-purple color is produced.

(2) Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 1 mg (potency) of idarubicin hydrochloride according to the labeled amount, in 1 mL of water, and add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 250 and 254 nm, between 285 and 289 nm, between 480 and 484 nm, and between 510 and 520 nm.

pH The pH of a solution obtained by dissolving an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of idarubicin hydrochloride, in 5 mL of water is between 5.0 and 7.0.

Purity ***Clarity and color of solution***—Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of idarubicin hydrochloride according to the labeled amount, in 5 mL of water: the solution is clear and yellow-red in color.

Water Not more than 4.0 % (0.3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 8.9 EU/mg (potency) of idarubicin hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injectsion It meets the requirement.

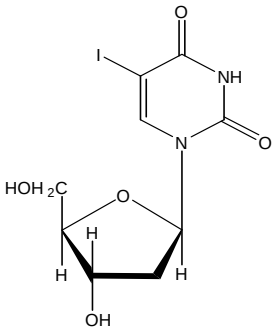
Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 container of Idarubicin Hydrochloride for Injection add the mobile phase containing no sodium lauryl sulfate to make *V* mL so that each mL contains 0.2 mg (potency) of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), add the mobile phase containing no sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

$$\text{Amount [mg (potency)] of idarubicin hydrochloride } (C_{26}H_{27}NO_9 \cdot HCl) = \text{Amount [mg (potency)] of Idarubicin Hydrochloride RS} \times \frac{A_T}{A_S} \times \frac{V}{50}$$

Assay Proceed as directed in the Assay under Idarubicin Hydrochloride. Weigh accurately an amount of Idarubicin Hydrochloride for Injection, equivalent to about 5 mg (potency) according to the labeled potency, dissolve in the mobile phase containing no sodium lauryl sulfate to make exactly 25 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Idoxuridine



$C_9H_{11}IN_2O_5$: 354.10

1-[(2*R*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-iodo-1,2,3,4-tetrahydropyrimidine-2,4-dione
[*54-42-2*]

Idoxuridine, when dried, contains 98.0 % and not more than 101.0 % of idoxuridine ($C_9H_{11}IN_2O_5$).

Description Idoxuridine appears as colorless, crystals or a white, crystalline powder. Idoxuridine is odorless.
Idoxuridine is freely soluble in *N,N*-dimethylformamide, slightly soluble in water, very slightly soluble in ethanol (95) and practically insoluble in ether. Idoxuridine dissolves in sodium hydroxide TS.

Melting point—About 176 °C (with decomposition).

Identification (1) Dissolve 10 mg of Idoxuridine in 5 mL of water by warming, add 5 mL of diphenylamine-acetic acid (100) TS and heat for 5 minutes: a blue color is observed.

(2) Heat 0.1 g of Idoxuridine: a purple gas evolves.

(3) Dissolve 2 mg of Idoxuridine and Idoxuridine TS in 50 mL of 0.01 mol/L sodium hydroxide VS and determine the absorption specta of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelength .

Specific Optical Rotation $[\alpha]_D^{20}$: +28 ~ +31° (after drying, 0.20 g, sodium hydroxide TS, 20 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Idoxuridine in 5 mL of a solution of sodium hydroxide (1 in 200): the solution is clear and colorless.

(2) ***Iodine and iodide***—Dissolve 0.10 g of Idoxuridine in 20 mL of water and 5 mL of sodium hydroxide TS and add immediately 5 mL of dilute sulfuric acid under ice-cooling. Allow to stand for 10 minutes with occasional shaking, and filter. Transfer the filtrate into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100), shake for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution—Weigh accurately 0.111 g of potassium iodide and dissolve in water to make 1000 mL. To exactly 1 mL of this solution, add 19 mL of water, 5 mL of sodium hydroxide TS and 5 mL of dilute sulfuric acid, mix and filter. Transfer the filtrate to a Nessler tube and proceed in the same manner.

(2) ***Heavy metals***—Proceed with 2.0 g of Idoxuridine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve about 0.10 g of Idoxuridine in exactly 10 mL of a mixture of dilute ethanol and ammonia solution (28) (99 : 1) and use this

solution as the test solution. Perform the test with the test solution as directed under the Thin-layer Chromatography. Spot 50 μL of the test solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diluted 2-propanol (2 in 3) (4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than principal spot appears.

Loss on Drying Not more than 0.5 % (2 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.3 % (1 g).

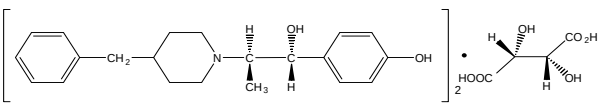
Assay Weigh accurately about 0.7 g of Idoxuridine, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 35.410 mg of $C_9H_{11}IN_2O_5$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ifenprodil Tartrate



$(C_{21}H_{27}NO_2)_2{\cdot}C_4H_6O_6$: 800.98

4-[2-(4-Benzylpiperidin-1-yl)-1-hydroxypropyl]phenol; 2,3-dihydroxybutanedioic acid [*23210-58-4*]

Ifenprodil Tartrate contains not less than 98.5 % and not more than 101.0 % of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2{\cdot}C_4H_6O_6]$, calculated on the anhydrous basis.

Description Ifenprodil Tartrate is a white crystalline powder and is odorless.

Ifenprodil Tartrate is freely soluble in acetic acid (100), soluble in ethanol (95), slightly soluble in water or in methanol and practically insoluble in ether.

Specific Optical Rotation—$[\alpha]_D^{20}$: +11~ +15° (1.0 g, calculated on the anhydrous basis, ethanol (95), 20 mL, 100 mm).

Melting point—About 148 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Ifenprodil Tartrate and Ifenprodil Tartrate RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelenght.

(2) Determine the infrared spectra of Ifenprodil Tartrate and Ifenprodil Tartrate RS as directed in the potassium bromide disc method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.4 g of Ifenprodil Tartrate in 40 mL of water by warming. After cooling, add 0.5 mL of ammonia TS to this solution, extract with two 40-mL volumes of chloroform and collect the water layer. Evaporate 30 mL of the water layer on a water-bath to dryness and after cooling, dissolve the residue in 6 mL of water: the solution responds to the Qualitative Tests for tartrate.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Ifenprodil Tartrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.30 g of Ifenprodil Tartrate in 10 mL of diluted ethanol (3 in 4) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted ethanol (3 in 4) to make exactly 200 mL and use this solution as the standard solution. Perform the test solution with the test and standard solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, 1-butanol and ammonia solution (28) (140 : 40 : 20 : 1) to a distance of about 10 cm and air-dry the plate. Spray hexachloroplatinic (IV) acid-potassium iodide TS evenly on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water Not more than 4.0 % (0.5 g, volume titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

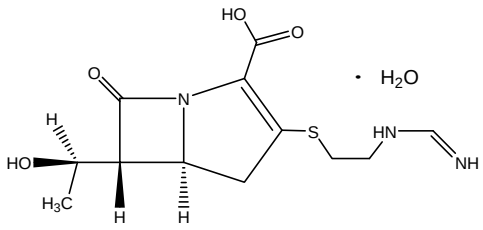
Assay Weigh accurately about 0.5 g of Ifenprodil Tartrate, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Determination Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.05 mg of $(C_{21}H_{27}NO_2)_2{\cdot}C_4H_6O_6$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Imipenem Hydrate



$C_{12}H_{17}N_3O_4S \cdot H_2O$: 317.36

(5*R*,6*S*)-3-({2-[(*E*)-(aminomethylidene)amino]ethyl}sulfanyl)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid [*54-42-2*]

Imipenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg of imipenem ($C_{12}H_{17}N_3O_4S$: 299.35), calculated on the anhydrous basis.

Description Imipenem Hydrate appears as white to pale yellow crystalline powder.
Imipenem Hydrate is sparingly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Imipenem Hydrate and Imipenem RS in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the absorption spectra of Imipenem Hydrate and Imipenem RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +89 ~ +94° (50 mg calculated on the anhydrous basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0), 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Imipenem Hydrate in 200 mL of water is between 4.5 and 7.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Imipenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Put 2.0 g of Imipenem Hydrate in a crucible, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes evolve. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure once more. Then add 2 mL of hydrogen peroxide, heat, and repeat this procedure until the color of the solution changes to colorless to pale yellow. After cooling, heat again until white fumes evolve. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 1 ppm).

(3) ***Related substances***—Weigh accurately about 50 mg of Imipenem Hydrate, dissolve in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the peak area of thienamycin from the test solution is not larger than 1.4 times the peak area of imipenem from the standard solution, and each peak area other than imipenem and thienamycin from the test solution is not larger than 1/3 times the peak area of imipenem from the standard solution. The total area of the peaks other than imipenem and thienamycin from the test solution is not larger than the peak area of imipenem from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of the standard solution, and add 0.1 mol/L 3-(*N*-morpholino) propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL. Confirm that the peak area of imipenem obtained from 10 μL of this solution is equivalent to 7 to 13 % of the peak area of imipenem from the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of imipenem is not more than 2.0 %.

Relative retention time: The relative retention time of thienamycin with respect to imipenem is about 0.8.

Time span of measurement: About 2 times as long as the retention time of imipenem

Water 5.0 ~ 8.0 % (20 mg, coulometric titration, water evaporation temperature: 140 °C).

Sterility Test It meets the requirement, when Imipenem Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.17 EU/mg (poten-

cy) of imipenem, when Imipenem Hydrate is used in a sterile preparation.

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 50 mg (potency) each of Imipenem Hydrate and Imipenem RS, dissolve each in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of imipenem. Perform the test within 30 minutes after preparation of the test solution and standard solution.

$$\text{Amount [μg (potency)] of imipenem } (C_{12}H_{17}N_3O_4S) = \text{Amount [μg (potency)] of Imipenem RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) and acetonitrile (100 : 1)

Flow rate: Adjust the flow rate so that the retention time of imipenem is about 6 minutes.

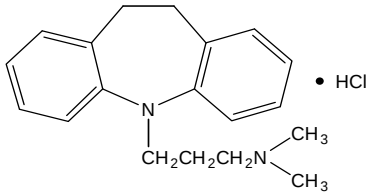
System suitability

System performance: Weigh accurately about 50 mg of Imipenem Hydrate and about 75 mg of resorcinol, and dissolve in exactly 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0). When the procedure is run with 10 μL of this solution under the above operating conditions, imipenem and resorcinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of imipenem is not more than 0.80 %.

Containers and Storage ***Containers***—Hermetic containers.

Imipramine Hydrochloride



$C_{19}H_{24}N_2 \cdot HCl$: 316.87

3-(10,11-Dihydro-5*H*-dibenzo[b,f]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine Hydrochloride [*113-52-0*]

Imipramine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$).

Description Imipramine Hydrochloride is a white to pale yellowish white, crystalline powder and is odorless.

Imipramine Hydrochloride is freely soluble in water or in ethanol (95) and practically insoluble in ether.

Imipramine Hydrochloride is gradually affected by light.

Identification (1) Dissolve 5 mg of Imipramine Hydrochloride in 2 mL of nitric acid: a deep blue color is observed.

(2) Dissolve 5 mg of Imipramine Hydrochloride and Imipramine Hydrochloride TS in 250 mL of 0.01 mol/L hydrochloric acid TS and determine the absorption spectra of these solution as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve about 50 mg of Imipramine Hydrochloride in 5 mL of water, add 1 mL of ammonia TS, allow to stand for 5 minutes, filter and acidify the filtrate with dilute nitric acid: it responds to the Qualitative Tests (2) for chloride.

Melting Point 170 ~ 174 °C (with decomposition).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Imipramine Hydrochloride in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—Take exactly 1.0 mL of cobalt (II) chloride hexahydrate colorimetric stock solution, 2.4 mL of iron (III) chloride hexahydrate CS, 0.4 mL of cupric sulfate colorimetric stock solution and 6.2 mL of diluted hydrochloric acid (1 in 40) and mix. Pipet 0.5 mL of this solution and add exactly 9.5 mL of water.

(2) ***Heavy metals***—Proceed with 1.0 g of Imipramine Hydrochloride according to Method 2, and per-

form the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Iminodibenzyl*—**Dissolve 50 mg of Imipramine Hydrochloride in 10 mL of a mixture of hydrochloric acid and ethanol (95) (1 : 1) in a brown volumetric flask. Cool the flask in ice-water, add 5 mL of an ethanol (95) solution of furfural (1 in 250) and 5 mL of hydrochloric acid and allow to stand at 25 °C for 3 hours. Add a mixture of hydrochloric acid and ethanol (95) (1 : 1) to make 25 mL and determine the absorbance of this solution at 565 nm as directed under Ultraviolet-visible Spectrophotometry: not more than 0.16.

(4) ***Related substances*—**Dissolve 0.20 g of Imipramine Hydrochloride in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of this solution and add ethanol (95) to make exactly 50 mL. Pipet 5.0 mL of this solution, add ethanol (95) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11 : 7 : 1 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105°C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Imipramine Hydrochloride, previously dried and dissolve in 20 mL of water. Add 5 mL of sodium hydroxide TS and extract with three 20 mL volumes of chloroform. Filter each extract through a pledget of absorbent cotton on which a small quantity of anhydrous sodium sulfate is placed. Combine the chloroform extracts and titrate with 0.1 mol/L perchloric acid VS until the yellow solution changes to red-purple (indicator: 10 drops of metanil yellow TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.687 mg of $C_{19}H_{24}N_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Imipramine Hydrochloride Tablets

Imipramine Hydrochloride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of the imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$: 316.87).

Method of Preparation Prepare as directed under Tablets, with Imipramine Hydrochloride.

Identification (1) Weigh a quantity of powered Imipramine Hydrochloride Tablets, equivalent to 0.25 g of Imipramine Hydrochloride according to the labeled amount, add 25 mL of chloroform and filter after shaking well. Evaporate the filtrate on a water-bath. Proceed with the residue as directed in the Identification (1) under Imipramine Hydrochloride.

(2) Dissolve an amount of the residue obtained in (1), equivalent to 5 mg of Imipramine Hydrochloride, in 250 mL of 0.01 mol/L hydrochloric acid TS and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 249 nm and 253 nm and a shoulder between 270 nm and 280 nm.

(3) Dry the residue obtained in (1) at 105 ºC for 2 hours: the residue melts between 170 ºC and 174 ºC (with decomposition)

Dissolution Test Perform the test with 1 tablet of Imipramine Hydrochloride Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using pH 6.8 phosphate buffer solution (1 in 2) as the dissolution solution. Take more than 20 mL of the dissolved solution after 60 minutes from the start of the dissolution test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the diluted pH 6.8 phosphate buffer solution (1 in 2) to make exactly V' mL so that each mL of the filtrate contains about 10 μg of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately 0.025 g of Imipramine Hydrochloride RS, previously dried at 105 °C for 2 hours, dissolve in the diluted pH 6.8 phosphate buffer solution (1 in 2) to make exactly 100 mL. Pipet 4.0 mL of this solution, add the 1st fluid to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at the wavelength of 250 nm as directed under Ultraviolet-visible Spectrophotometry, respectively.

The dissolution rate of Imipramine Hydrochloride Tablets in 60 minutes should be not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 36$$

W_S : Amount (mg) of Imipramine Hydrochloride RS,

C: Labeled amount (mg) of imipramine hydrochlo-

ride ($C_{19}H_{24}N_2$·HCl) in 1 tablet.

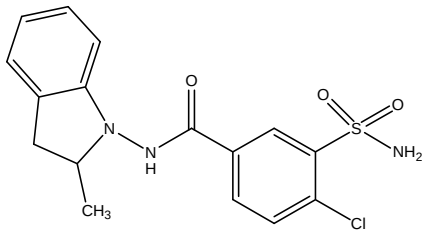
Uniformity of Dosage Units It meets the requirement.

Assay Take 20 Imipramine Hydrochloride Tablets, add exactly 200 mL of 0.01 mol/L hydrochloric acid TS and shake well until the tablets are completely disintegrated. After centrifuging the solution, pipet a volume of the clear superantant liquid, equivalent to about 0.025 g of imipramine hydrochloride ($C_{19}H_{24}N_2$·HCl) according to the labeled amount, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.025 g of Imipramine Hydrochloride RS, previously dried at 105 °C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL and use this solution as the standard solution. Pipet 3.0 mL each of the test solution and the standard solutions into separators which contain 15 mL of potassium biphthalate buffer solution, pH 5.6, 8 mL of bromocresol green-sodium hydroxide TS and 30 mL of chloroform and shake. Filter the chloroform layer through a pledget of absorbent cotton into a 100 mL volumetric flask. Repeat the extraction with two 30-mL portions of chloroform, combine the chloroform layers in the 100-mL volumetric flask and add chloroform to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution obtained by proceeding with 3 mL of 0.01 mol/L hydrochloric acid TS in the same manner as blank. Determine the absorbances, A_T and A_S, for the test solution and the standard solution, respectively, at 416 nm .

$$\text{Amount (mg) of imipramine hydrochloride } (C_{19}H_{24}N_2\cdot HCl) = \text{Amount (mg) of Imipramine Hydrochloride RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Indapamide



$C_{16}H_{16}ClN_3O_3S$: 365.84

4-Chloro-*N*-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoylbenzamide [*26807-65-8*]

Indapamide contains not less than 98.0 % and not more than 101.0 % of indapamide ($C_{16}H_{16}ClN_3O_3S$), calculated on the dried basis.

Description Indamaide is a white, crystalline powder. Indapamide is freely soluble in ethanol (99.5), and practically insoluble in water.
A solution of Indapamide in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Indapamide and Indapamide RS in methnol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Indapamide and Indapamide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Indapamide as directed under Flame Coloration Test: a green color appears.

Melting Point 167 ~ 171 °C.

Purity (1) ***Chloride***—Dissolve 1.5 g of Indapamide in 50 mL of water, shake for 15 minutes, allow to stand in an ice bath for 30 minutes, and filter. To 30 mL of the filtrate add 6 mL of dilute actic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid (not more than 0.01 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Indapamide according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Perform the test without exposure to daylight. Dissolve about 0.3 g of Indapamide in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 30 mg of Indapamide RS in methanol to make exactly 100 mL, and use this solution as the standard solution (1). Add methanol to 10 mL of the standard solution (1) to make exactly 20 mL, and use this solution as the standard solution (2). And, add methanol to 10 mL of the standard solution (2) to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spots separately, 10 μL of the test solution, 10 μL each of the standard solution (2) and the standard solution (3) on a plate of silica gel with fluorescent indicator for the thinlayer chromatography. Develop the plate with a mixture of toluene, ethyl acetate and acetic acid (100) (70:30:1) to a distance of about 15 cm. Remove the plate from the developing chamber, and air-dry the plate. Examine under ultraviolet light(main wavelength: 254 μm), and compare the intensities of any secondary spots ob-

served in the chromatogram of the test solution with those of the principal spots in the chromatograms of the standard solution: secondary spot from the chromatograms of the test solution is not larger or more intense than the principal spot obtained from standard solution (2) (0.5 %), and the sum of the intensities of the secondary spots obtained from the test solution is not more than 2.0 %.

Loss on Drying Not more than 3.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, P_2O_5, 110 °C, 2 hours).

Residue on Ignition Not more than 0.1 %.

Assay Weigh about 0.1 g of Indapamide, accurately weighed, dissolve in 5.0 mL of the internal standard solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Indapamide RS, dissolve in 1.0 mL of internal standard solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under the Liquid chromatography according to the following operating conditions. Determine the ratios of peak areas of Indapamide, Q_T and Q_S to the peak area of the internal standard substance of each solution.

$$\text{Amount (mg) of indapamide } (C_{16}H_{16}ClN_3O_3S) = \text{Amount (mg) of Indapamide RS} \times \frac{Q_T}{Q_S} \times 5$$

Internal standard solution—Weigh accurately 50 mg of *p*-chloroacetanilide, dissolve in methanol to make exactly 10 mL.

Operating conditions

Detector : An ultraviolet absorption photometer (wave length: 254 nm).

Column : A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with between 3 and 10 μm in particle diameter octadecylsilanized silica gel for liquid chromatography.

Mobile phase : A mixture of water, acetonitrile, methanol and acetic acid (100) (650:175:175:1).

Flow rate: 2.0 mL per minute.

System suitability

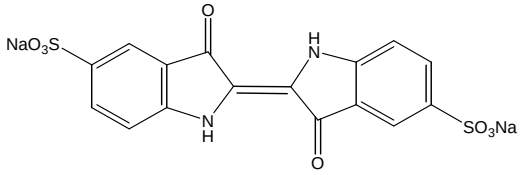
System performance: When the procedure is run with 5 μL of the standard solution, the resolution of *p*-chloroacetanilide peak and Indapamide peak is not less than 2.0, and symmetry factor of indapamide peak is not more than 2.0.

System repeatability: When the test is repeated 5 times with 5 μL each of the standard solution under the above operating conditions: the relative standard deviation of the peak area of indapamide is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Indigocarmine



$C_{16}H_8N_2Na_2O_8S_2$: 466.35

Disodium (2*E*)-3-oxo-2-(3-oxo-5-sulfonato-1*H*-indol-2-ylidene)-1*H*-indole-5-sulfonate [*860-22-0*]

Indigocarmine, when dried, contains not less than 95.0 % and not more than 101.0 % of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$).

Description Indigocarmine appears as blue to dark blue powder or granules and is odorless.
Indigocarmine is sparingly soluble in water and practically insoluble in ethanol (95) or in ether.
Indigocarmine is hygroscopic.
When compressed, Indigocarmine has a coppery luster.

Identification (1) A solution of Indigocarmine (1 in 100) is dark blue in color. Perform the following tests with this solution as the test solution: the dark blue color of each solution disappears.

(i) Add 1 mL of nitric acid to 2 mL of the test solution.

(ii) Add 1 mL of bromine TS to 2 mL of the test solution.

(iii) Add 1 mL of chlorine TS to 2 mL of the test solution.

(iv) Add 2 mL of sodium hydroxide TS and 0.2 g of zinc dust to 2 mL of the test solution and warm.

(2) Dissolve 0.1 g of Indigocarmine and Indigocarmine RS in 100 mL of an ammonium acetate solution (1 in 650). To 1 mL each of these solutions, add an ammonium acetate solution (1 in 650) to make 100 mL and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibits similar intensitie of absorption at the same wavenlenght.

(3) Ignite 1 g of Indigocarmine to carbonize. After cooling, add 20 mL of water to the residue, shake and filter the mixture: the filtrate responds to the Qualitative Tests for sodium salt and for sulfate.

pH Dissolve 0.10 g of Indigocarmine in 20 mL of water: the pH of the solution is between 5.0 and 6.0.

Purity (1) ***Water-insoluble substances***—Take 1.00 g of Indigocarmine, add 200 mL of water, shake and

filter through a tared glass filter (G4). Wash the residue with water until the blue color of the filtrate becomes practically colorless and dry the residue at 105 ºC for 4 hours: the weight of the residue does not exceed 5.0 mg.

(2) ***Lead***—Place 4.0 g of Indigocarmine in a Kjeldahl flask, moisten with water, and add 10 mL of sulfuric acid and 5 mL of nitric acid. As soon as the first violent reaction subsides, heat until most of the brown fumes are expelled. Repeat the addition of 1 to 3 mL of nitric acid at a time, and heat until the Indigocarmine is almost decomposed and most of the organic matter is in solution. Then add, cautiously and in small portions, 5 mL of perchloric acid. When the violent reaction subsides, continue the addition of small amounts of nitric acid, and heat until a colorless solution is obtained. If the solution does not become clear in 10 to 20 minutes after the addition of the perchloric acid, add a further 1 to 3 mL of perchloric acid, and add nitric acid until the solution becomes colorless. Boil this solution for 10 to 15 minutes, cool, and neutralize with 1 mol/L sodium hydroxide. Transfer this solution to a 100 mL volumetric flask, add water to make 100 mL, and use this solution as the test solution. Put 5 mL of the test solution in a separatory funnel, wash with 10 mL of water, add 3 mL of a solution of diammonium hydrogen citrate, 0.5 mL of hydroxylamine hydrochloride TS and 2 drops of phenol red TS, and alkalify with ammonia solution (28). Cool the solution if necessary, add 1 mL of a solution of potassium cyanide, extract with 5 mL volumes of dithizone solution for extraction until the extract shows a green color, and combine the extracts in another separatory funnel. To the combined extracts add 20 mL of diluted nitric acid (1 in 100), shake for 30 minutes, and discard the chloroform layer. To the nitric acid layer add 5.0 mL of standard dithizone solution and 4 mL of ammonia-cyanide TS, and shake for 30 seconds: the purple color of the chloroform layer is not more intense than the color obtained by proceeding with 10 mL of diluted standard lead solution in the same manner as the test solution (not more than 10 ppm).

Diluted standard lead solution—Pipet 5 mL of standard lead solution, and add diluted nitric acid (1 in 100) to make 50 mL.

(3) ***Arsenic***—Place 0.8 g of Indigocarmine in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid and ignite gently. Repeat the addition of 2 to 3 mL of nitric acid occasionally and continue to heat until a colorless to pale yellow solution is obtained. After cooling, add 15 mL of a saturated ammonium oxalate solution, heat the solution until dense white fumes are evolved and concentrate to 2 to 3 mL. After cooling, dilute with water to make 10 mL and perform the test with 5 mL of this solution as the test solution (not more than 5 ppm).

Loss on Drying Not more than 10.0 % (1 g, 105 ºC, 2 hours).

Residue on Ignition 28.0 ~ 38.0 % (after drying, 1 g).

Assay Weigh accurately about 0.5 g of Indigocarmine, previously dried, add 15 g of sodium hydrogen tartrate monohydrate and dissolve in 200 mL of water, boil with bubbling of a stream of carbon dioxide and titrate, while being hot, with 0.1 mol/L titanium (III) chloride VS until the color of the solution changes from blue through yellow to orange.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.318 mg of $C_{16}H_8N_2Na_2O_8S_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Indigocarmine Injection

Indigocarmine Injection is an aqueous solution for injection. Indigocarmine Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$: 466.35).

Method of Preparation Prepare as directed under Injections, with Indigocarmine.

Description Indigocarmine Injection is a dark blue liquid.

pH—3.0 ~ 5.0.

Identification (1) Take a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of nitric acid: the dark blue color of the liquid disappears and a yellow-brown color develops.

(2) Take a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of bromine TS: the dark blue color disappears and a yellow-brown color develops.

(3) Take a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of chlorine TS: the dark blue color disappears and a yellow-brown color develops.

(4) Take a volume of Indigocarmine Injection, equivalent to 0.01 g of Indigocarmine according to the labeled amount, add ammonium acetate solution (1 in 650) to make 1000 mL and determine the absorbance of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 610 nm and 614 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 7.5 EU/mg of indigocarmine.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement

Determination of Volume of Injection in Containers It meets the requirement.

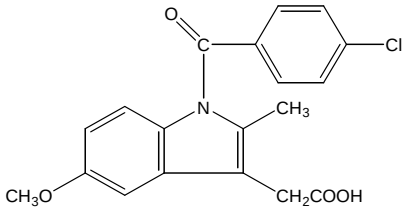
Assay Measure exactly a volume of Indigocarmine Injection, equivalent to about 0.2 g of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$), add 6 g of sodium bitartrate and dissolve in water to make 200 mL. Then boil under a carbon dioxide stream and proceed as directed in the Assay under Indigocarmine.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.318 mg of $C_{16}H_8N_2Na_2O_8S_2$

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Indomethacin



$C_{19}H_{16}ClNO_4$: 357.79

2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid [*53-86-1*]

Indometacin, when dried, contains not less than 98.0 % and not more than 101.0 % of indometacin ($C_{19}H_{16}ClNO_4$).

Description Indometacin is a white to pale yellow, very fine crystalline powder.
Indometacin is sparingly soluble in methanol, in ethanol (95) or in ether and practically insoluble in water.
Indometacin dissolves in sodium hydroxide TS.
Indometacin is colored by light
Melting point—155 ~ 162 ºC.

Identification (1) Dissolve 2 mg of Indomethacin in 100 mL of methanol and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 317 nm and 321 nm.

(2) Determine the infrared spectra of Indometacin and Indometacin RS, as directed in the potassium bromide disc method under nfrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, recrystallize the test and the RS with ether, filter and dry the crystals and perform the test with the crystals.

(3) Perform the test with Indomethacin as directed under the Flame Coloration Test (2): a green color appears.

Purity (1) ***Acid***—Take 1.0 g of Indomethacin, add 50 mL of water, shake for 5 minutes and filter. To the filtrate, add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: a red color develops.

(2) ***Heavy metals***—Proceed with 1.0 g of Indometacin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Indomethacin according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Indomethacin in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution and add methanol to make exactly 50 mL. Pipet 5.0 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution, Perform the test with the test solution and the standard solutions as directed under the Thin-layer Chromatography. Spot 25 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated ether and acetic acid (100) (100 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.7 g of Indomethacin, previously dried, dissolve in 60 mL of methanol, add 30 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 35.779 mg of $C_{19}H_{16}ClNO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Indomethacin Capsules

Indomethacin Capsules contain not less than 90.0 % and not more than 110.0 %of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$: 357.79).

Method of Preparation Prepare as directed under Capsules, with Indometacin.

Identification Powder the contents of Indomethacin Capsules. Take a portion of the powder, equivalent to 0.1 g of Indomethacin according to the labeled amount, add 20 mL of chloroform, shake well and centrifuge. Filter the clear supernatant liquid and evaporate the filtrate to dryness. After cooling, dissolve the residue in 20 mL of methanol. To 10 mL of this solution, add methanol to make 50 mL, then to 2 mL of this solution, add methanol to make 100 mL and use this solution as the test solution. Determine the absorption spectrum of the test solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 317 nm and 321 nm.

Purity ***Related substances*—**Powder the content of Indomethacin Capsules. Take a portion of the powder, equivalent to 0.10 g of Indometacin according to the labeled amount, add exactly 10 mL of methanol, shake well, filter and use the filtrate as the test solution. Dissolve 0.025 g of Indometacin RS in methanol to make exactly 50 mL. Pipet 1.0 mL of the solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Proceed as directed in the Purity (4) under Indomethacin.

Dissolution Test Take 1 capsule of Indomethacin Capsules and perform the test using 900 mL of a mixture of water and phosphate buffer solution, pH 7.2, (4 : 1) as the dissolution solution at 100 revolutions per minute as directed in Method 1 under the Dissolution Test. Take 20 mL or more of the dissolved solution at 20 minutes after starting the test and filter through a membrane filter (less than 0.8 nm in pore size). Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 0.03 g of Indomethacin RS, previously dried at 105 ºC for 4 hours, dissolve in a mixture of water and phosphate buffer solution, pH 7.2, (4 : 1) to make exactly 1000 mL and use this as the standard solution, Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 320 nm as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Indometacin Capsules in 20 minutes should be not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{90}{C}$$

W_S: Amount (mg) of Indomethacin RS.

C: Labeled amount (mg) of indomethacin ($C_{19}H_{16}ClNO_4$) in 1 capsule.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 20 Indomethacin Capsules. Powder the combined contents and weigh accurately a portion of the powder, equivalent to about 0.05 g of indomethacin ($C_{19}H_{16}ClNO_4$). Dissolve in 40 mL of methanol and add methanol to make exactly 50 mL. Filter this solution, discarding the first 10 mL of the filtrate. Pipet the subsequent 5.0 mL of the filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.05 g of Indomethacin RS, previously dried at 105 ºC for 4 hours and dissolve in methanol to make exactly 50 mL. Pipet 5.0 mL of the solution, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Indometacin to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of indomethacin } (C_{19}H_{16}ClNO_4) = \text{Amount (mg) of Indomethacin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7 : 3).

Flow rate: Adjust the flow rate so that the retention time of Indometacin is about 8 minutes.

System suitability

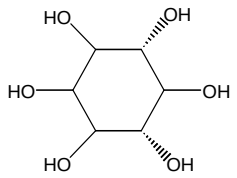
System performance: Dissolve 50 mg of 4-chhlorobenzoic acid, 30 mg of butyl *p*-hydroxybenzoate and 50 mg of Indometacin in 50 mL of methanol. Take 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with

20 μL of this solution, as directed under the above operating conditions, 4-chlorobenzoic acid, butyl *p*-hydroxybenzoate and indomethacin are eluted in this order, with the resolution between the peaks of 4-chlorobenzoic acid and butyl *p*-hydroxybenzoate being not less than 2.0 and between the peaks of butyl *p*-hydroxybenzoate and indomethacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution, as directed under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Inositol



Inosit $C_6H_{12}O_6$: 180.16

Cyclohexane-1,2,3,4,5,6-hexol [*87-89-8*]

Inositol, when dried, contains not less than 97.0 % and not more than 101.0 % of inositol ($C_6H_{12}O_6$).

Description Inositol appears as white crystals or crystalline powder, is odorless and has a sweet taste.
Inositol is very soluble in water, and practically insoluble in ethanol (95), in chloroform, or in ether.
Inositol shows no specific optical rotation.

pH—The pH of a solution of Inositol is neutral.

Identification (1) Add 6 mL of nitric acid to 1 mL of Inositol solution in water (1 in 50). Evaporate in a water-bath and add 0.5 mL of strontium nitrate solution (1 in 10) to the residue. Evaporate in a water-bath again: a red-purple color is observed.

(2) Add 1 mL of lead acetate TS to 4 mL of Inositol solution in water (1 in 100), shake and mix. Heat in a water-bath for 5 minutes: the solution changes to a half-transparent gel.

(3) Weigh accurately about 0.2 g of Inositol, previously dried, transfer to a 250 mL beaker, add 5 mL of a mixture of 1 mL of dilute sulfuric acid and 50 mL of acetic anhydride, cover the beaker with a watch glass, heat in a water bath for 20 minutes, and cool in ice. Add 100 mL of water, boil for 20 minutes, cool, transfer the contents of the beaker to a 250 mL separatory funnel, and wash with a small amount of water. Extract with 30 mL, 25 mL, 20 mL, 15 mL, 10 mL, and 10 mL of chloroform, combine all of the chloroform extracts, and wash with 10 mL of water. Filter the chloroform layer through absorbent cotton, wash the water layer and absorbent cotton with 10 mL of chloroform, combine the filtrate and washings, evaporate to dryness in a water bath, dry at 105 °C for 2 hours, and cool. The melting point of the hexaacetyl inositol thus obtained is between 212 and 216 °C.

Melting Point 223 ~ 227 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve about 1.0 g of Inositol in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with about 2.0 g of Inositol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005 %).

(3) ***Sulfate***—Perform the test with about 4.0 g of Inositol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006 %).

(4) ***Lead***—Dissolve 20.0 g of Inositol in dilute acetic acid to make 100 mL. To this solution add 2 mL of a saturated solution of ammonium pyrrolidine dithiocarbamate and 10 mL of 4-methyl-2-pentanone, shake for 30 seconds, allow the layers to separate with protection from light, take the 4-methyl-2-pentanone layer, and use as the test solution. Separately, to 20.0 g each of Inositol add 0.5 mL, 1.0 mL, and 1.5 mL of standard lead solution, proceed in the same manner as the test solution, and use these solutions as the standard solutions. To 2 mL of a saturated solution of ammonium pyrrolidine dithiocarbamate add 10 mL of 4-methyl-2-pentanone, shake for 30 seconds, allow the layers to separate with protection from light, take the 4-methyl-2-pentanone layer, and use as the blank solution. Perform the test with the test solution, standard solutions, and blank solution as directed in the calibration curve method under Atomic Absorption Spectrophotometry, and calculate the concentration of lead in the test solution: not more than 0.5 ppm.

Gas: Dissolved acetylene – Air
Lamp: Lead hollow cathode lamp
Wavelength: 283.3 nm

(5) ***Barium***—Dissolve 10.0 g of Inositol in water to make 100 mL, pipet 10 mL of this solution, add 1 mL of dilute sulfuric acid, and allow to stand for 1 hour: the turbidity of the solution is not more intense than the following control solution.

Control solution—Dissolve 10.0 g of Inositol in water to make 100 mL, pipet 10 mL of this solution, and add 1 mL of distilled water.

(6) ***Iron***—Dissolve about 2.0 g of Inositol in 40 mL of water, add 2 mL of hydrochloric acid, 40 mg of ammonium peroxydisulfate and 2 mL of ammonium

thiocyanate TS: the solution is not more intense than the following control solution.

Control solution—Prepare in the same manner, using 1.0 mL of standard iron solution instead of Inositol.

(7) ***Calcium***—Dissolve about 1.0 g of Inositol in 10 mL of water, add 1 mL of ammonium oxalate TS and allow to stand for 1 minute: the solution is transparent.

(8) ***Reducible substance to Fehling's TS***—Dissolve about 0.5 g of Inositol in 10 mL of water, add 5 mL of Fehling's TS, boil for 3 minutes and allow to stand for 30 minutes: no orange to red precipitate is observed.

(9) ***Related substances***—Weigh accurately 0.5 g of Inositol, dissolve in water to make 10 mL, and use this solution as the test solution. Separately, weigh accurately 0.5 g of Inositol RS, dissolve in water to make 10 mL, and use this solution as the standard stock solution. Pipet 2.0 mL of this solution, dissolve in water to make 100 mL so that each mL contains about 1 mg, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the amount of related substaces: the amount of each related substance is not more than 0.3 %, and the total amount of related substances is not more than 1.0 %. Exclude any related substance less than 0.05 %.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration (mg/mL) of inositol in the standard solution

C_T: Concentration (mg/mL) of Inositol in the test solution

A_i: Peak area of each related substance obtained from the test solution

A_S: Peak area of inositol obtained from the standard solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Weigh accurately suitable amounts of Inositol RS and D-mannitol, dissolve in water to that each mL contains 0.05 mg, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the relative retention time of D-mannitol with respect to inositol is about 1.3 with the resolution between these peaks being not less than 4.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard stock solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 ours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g each of Inositol and Inositol RS, dissolve each in water to make exactly 10 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of inositol.

$$\text{Amount (mg) of inositol } (C_6H_{12}O_6) = \text{Amount (mg) of Inositol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: A differential refractometer (a constant temperature of about 30 to 35 °C)

Column: A stainless steel column or equivalent about 7.8 mm in internal diameter and about 30 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (Ca type) composed with a sulfonated styrene-divinylbenzene copolymer (9 μm in particle diameter).

Column temperature: A constant temperature of about 85 °C

Mobile phase: Water

Flow rate: 0.5 mL/minute

System suitability

System performance: Weigh accurately suitable amounts of Inositol RS and D-mannitol, dissolve in water so that each mL contains 0.05 mg, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention time of D-mannitol with respect to inositol is about 1.3 with the resolution between these peaks being not less than 4.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard stock solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Insulin

Insulin is obtained from the pancreas of healthy bovine or porcine, that has blood sugar-decreasing activity.

Potency of Insulin, calculated on the dried basis, is not less than 26 Insulin units in each mg. Insulin is labeled to indicate the animal species from which it is derived.

Description Insulin is a white, crystalline powder and is odorless.
Insulin is practically insoluble in water, in ethanol (95) or in ether.
Insulin dissolves in diluted hydrochloric acid (1 in 360) or in ether.
Insulin is hygroscopic.

Identification Dissolve 0.01 g of Insulin in 10 mL of 0.1 mol/L hydrochloric acid TS and use this solution as the test solution. Proceed as the Identification for Insulin Injection with the test solution.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Insulin in 10 mL of diluted hydrochloric acid (1 in 360): the solution is clear and colorless to pale yellow.

(2) ***Related substances***—Place 7.5 mg of Insulin in a suitable capped vial, and add 2.0 mL of 0.01 mol/L hydrochloric acid. Cap the vial, and shake gently. Use this solution within 2 hours when kept at room temperature or within 12 hours when kept in a refrigerator. Separately, weigh accurately 37.5 mg of Insulin RS, add 0.01 mol/L hydrochloric acid to make 10 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add 0.01 mol/L hydrochloric acid to make 10 mL, and use this solution as the standard solution (2). Pipet 1 mL of the standard solution (2), add 0.01 mol/L hydrochloric acid to make 10 mL, and use this solution as the standard solution (3). The standard solutions may be kept at room temperature for up to 12 hours or in a refrigerator for up to 48 hours. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_I and A_D, of insulin and A-21 desamido insulin, respectively, and A_S, the sum of the peak areas, by the automatic integration method. Calculate the amount of each related substance by the area percentage method: A-21 desamido insulin is not more than 10.0 %, and the total amount of related substances other than insulin and A-21 desamido insulin is not more than 5.0 %. For insulin derived from a single species, determine the peaks corresponding to bovine or porcine insulin: the amount of cross-contamination is not more than 1.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table. Adjust the mobile phase composition to obtain a retention time of about 31 minutes for insulin, with the A-21 desamido insulin eluting at the start of the concentration gradient.

Mobile phase A: A mixture of a solution prepared by dissolving 28.4 mg of anhydrous sodium sulfate in 1000 mL of water, adding 2.7 mL of phosphoric acid, and adjusting the pH to 2.3 with 2-aminoethanol if necessary, and acetonitrile (82 : 18)

Mobile phase B: A mixture of a solution prepared by dissolving 28.4 mg of anhydrous sodium sulfate in 1000 mL of water, adding 2.7 mL of phosphoric acid, and adjusting the pH to 2.3 with 2-aminoethanol if necessary, and acetonitrile (50 : 50)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	81	19
0-60	81	19
60-85	81→36	19→64
85-91	36	64
91-92	36→81	64→19

Flow rate: 1 mL/minute

System suitability

Test for required detectability: Proceed with 20 μL each of the standard solutions (1), (2), and (3) under the above operating conditions, determine the peak areas, A_A, A_B, and A_C, of the standard solutions (1), (2), and (3), respectively, and perform the calculations by following equations (1) and (2): confirm that the values are between 0.91 and 1.09 and between 0.7 and 1.3, respectively.

$$(1)\quad 10 \times \frac{A_B}{A_A}$$

$$(2)\quad 10 \times \frac{A_C}{A_A}$$

System performance: Dissolve about 1.5 mg of Insulin in 1.0 mL of 0.01 mol/L hydrochloric acid. Allow this solution to stand at room temperature for not more than 3 days to obtain a solution containing not less than 5 % of A-21 desamido insulin, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of insulin and A-21 desamido insulin is not less than 2.0, and the symmetry factor of the peak of insulin is not more than 1.8.

Loss on Drying Not more than 10.0 % (0.2 g, 105 °C, 16 hours).

Residue on Ignition Weigh accurately 0.02 to 0.04 g of Insulin in a tared platinum dish, add 2 drops of nitric

acid and heat the dish at first very gently and then strongly to incinerate. Place the dish in a muffle furnace, heat at 600°C for 15 minutes, cool in a desiccator (silica gel) and weigh: the weight of the residue is not more than 2.5 %.

Bacterial Endotoxins Less than 10 EU/mg of insulin.

Microbial Limit The total aerobic microbial count is not more than 300 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g and *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

Zinc Content Weigh accurately about 0.01 g of Insulin, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS and water to make exactly 50 mL. If necessary, dilute the solution with water so as to contain 0.4 to 1.0 μg of zinc (Zn: 65.41) per mL and use this solution as the test solution. Add water to an accurately measured volume of standard zinc solution for Atomic Absorption Spectrophotometry to make a solution containing 0.3 to 1.2 μg of zinc (Zn: 65.41) per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions and determine the amount of zinc in the test solution using the calibration curve obtained from the absorbance of the standard solution: the amount of zinc is not less than 0.27 % and not more than 1.08 %, calculated on the dried basis.

Gas: Dissolved acetylene - Air
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

Nitrogen Content Weigh accurately about 0.02 g of Insulin, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS and perform the test as directed under the Nitrogen Determination: not less than 14.5 % and not more than 16.5 % of nitrogen (N: 14.01) is found, calculated on the dried basis.

Assay (1) Animals: Select healthy rabbits weighing not less than 1.8 kg. Keep the rabbits in the laboratory not less than 1 week before use in the Assay by feeding them with an appropriate uniform diet and water.

(2) Diluent for insulin: Dissolve 1.0 to 2.5 g of phenol or *m*-cresol in 500 mL of 0.01 mol/L hydrochloric acid VS and add 14 to 18 g of glycerin and 0.01 mol/L hydrochloric acid VS to make 1000 mL.

(3) Standard stock solution: Weigh accurately about 0.02 g of insulin RS and dissolve in the diluent for Insulin to make a standard stock solution containing exactly 20.0 units in each mL. Preserve this solution between 1 °C and 15 °C and use within 6 months.

(4) Standard solution: Dilute two portions of the standard stock solution to make two standard solutions with the diluent for Insulin, one to contain exactly 2.0 units in each mL which is designated as the high-dose standard solution, S_H and the other to contain exactly 1.0 unit in each mL which is designated as the low-dose standard solution, S_L.

(5) Test solution: weigh accurately about 0.02g of Insulin according to the labeled units, dissolve with the diluent for insulin to make two different test solutions, one to contain exactly 2.0 units in each mL which is designated as the high-dose test solution, T_H and the other to contain exactly 1.0 unit in each mL which is designated as the low-dose test solution, T_L.

(6) Dose for injection: Select the dose for injection on the basis of trial or experience. Inject an identical volume, usually 0.3 to 0.5 mL, of the standard solutions and the test solutions throughout the whole run.

(7) Procedure: Divide the animals into 4 equal groups of not less than 6 animals each, with least difference in body weight. Withhold all food, except water, for not less than 14 hours before the injections and withhold water during the Assay until the final blood test is taken. Handle the animals with care in order to avoid undue excitement. Inject into each of the animals subcutaneously the dose of the standard solutions and the test solutions indicated in the following design.

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

The second injection should be made on the day after the first injection or within 1 week, using the dose of the standard solutions and the test solutions indicated in the following design.

First group	T_L	Third group	S_L
Second group	T_H	Fourth group	S_H

At 1 hour and 2.5 hours after the time of injection, obtain a sufficient blood to perform the test from a marginal ear vein of each animal and determine the blood sugar content of the blood samples according to (8).

Conversion Table for the blood sugar content (%)

mL*	0	1	2	3	4	5	6	7	8	9
0.0	0.385	0.382	0.379	0.376	0.373	0.370	0.367	0.364	0.361	0.356
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341	0.338	0.336	0.333
0.2	0.331	0.329	0.327	0.325	0.323	0.321	0.318	0.316	0.314	0.312

0.3	0.310	0.308	0.306	0.304	0.302	0.300	0.298	0.296	0.294	0.292
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278	0.276	0.274	0.272
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259	0.257	0.255	0.253
0.6	0.251	0.249	0.247	0.245	0.243	0.241	0.240	0.238	0.236	0.234
0.7	0.232	0.230	0.228	0.226	0.222	0.222	0.221	0.219	0.217	0.215
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202	0.200	0.199	0.197
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184	0.182	0.181	0.179
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166	0.164	0.163	0.161
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148	0.146	0.145	0.143
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131	0.119	0.127	0.125
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113	0.111	0.110	0.108
1.4	0.106	0.104	0.102	0.101	0.099	0.097	0.093	0.093	0.092	0.090
1.5	0.088	0.084	0.084	0.083	0.081	0.079	0.077	0.075	0.074	0.072
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059	0.057	0.056	0.054
1.7	0.052	0.050	0.048	0.047	0.045	0.043	0.041	0.039	0.038	0.036
1.8	0.034	0.032	0.031	0.029	0.027	0.025	0.024	0.022	0.020	0.019
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007	0.005	0.003	0.002

* Indicates the volume of 0.005 mol/L sodium thiosulfate VS required in titration. For example, if the amount was 1.28 mL, the blood sugar content would be 0.127% from the above table.

(8) Blood sugar determination: Place 5.0 mL of a solution of zinc sulfate (9 in 2000) in a test tube, 18 mm in outside diameter and 165 mm in length, add 1.0 mL of a solution of sodium hydroxide (1 in 250) and add gently 0.10 mL of the blood sample to the mixture in the test tube using a blood sugar pipet. Suck up the supernatant liquid into the pipet, wash out the remaining blood in the inner wall of the pipet and repeat this procedure. Shake thoroughly the contents in the test tube and heat the test tube in a water-bath for 3 minutes. Filter the mixture through a funnel, 30 to 40 mm in diameter in which a pledget of absorbent cotton, previously washed with two 3 mL volumes of warm water, has been placed, receive the filtrate into a test tube, 30 mm in internal diameter and 90 mm in length, wash the test tube and the funnel with two 3 mL volumes of water and combine the washings with the filtrate. Add 2.0 mL of alkaline potassium hexacyanoferrate (III) TS, heat in a water-bath for 15 minutes, cool immediately, add 3.0 mL of potassium iodide-zinc sulfate TS and 2.0 mL of diluted acetic acid (100) (3 in 100) and titrate the liberated iodine with 0.005 mol/L sodium thiosulfate VS (indicator: 2 to 4 drops of starch-sodium chloride TS). Perform a blank determination and make any necessary correction. From the consumed volume (mL) of 0.005 mol/L sodium thiosulfate VS, obtain the blood sugar content (%) according to the table of the previous page..

(9) Calculation: Sum up the two blood sugar values of each animal after each injection. Subtract the blood sugar value effected by the first injection from that effected by the second injection of each animal in the first group and the third group. The differences are symbolized as y_1 and y_3, respectively. Subtract the blood sugar value effected by the second injection from that effected by the first injection of each animal in the second group and the fourth group. The differences are symbolized as y_2 and y_4, respectively. Sum up not less than 6 values of individual differences in the blood sugar values, y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

$$\text{Units in each mg of Insulin} = \text{antilog M} \times \text{units in each mL of the high-dose standard solution} \times \frac{b}{a}$$

$$\text{M} = 0.301 \times \frac{Y_b}{Y_a}$$

$$Y_a = -Y_1+Y_2+Y_3-Y_4$$

$$Y_b = Y_1+Y_2+Y_3+Y_4$$

a: Mass (mg) of the sample,

b: Total volume (mL) of the high-dose test solution prepared by diluting the volume of the test with diluent for insulin.

Compute *L* (p = 0.95) by using the following equation: L should be not more than 0.1212. If *L* exceeds 0.1212,

repeat the Assay by increasing the number of animals or improving the Assay conditions in a better way until L becomes not more than 0.1212.

$$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

$$s^2 = \frac{\sum y^2 - \frac{Y}{f}}{n}$$

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f-1)$$

f: Number of the animals of each group.

Σy_2: Sum of spuares of y_1, y_2, y_3 and y_4 in each group.

t^2: Value shown in the following table against n for which s^2 is calculated.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 8 °C.

Insulin Injection

Insulin Injection is an aqueous solution for injection. Insulin Injection contains not less than 95.0 % and not more than 105.0 % of the labeled Insulin units.

Method of Preparation Suspend Insulin in water for injection, dissolve by adding hydrochloric acid and prepare as directed under Injections. Insulin Injection contains 0.10 to 0.25 g of phenol or cresol and 1.4 to 1.8 g of concentrated glycerin for each 100 mL of Insulin Injection. Insulin Injection should not contain sodium chloride.

Description Insulin Injection is a clear, colorless or pale yellow liquid.

Identification Adjust Insulin Injection to pH between 5.1 and 5.3 with a solution of sodium hydroxide (1 in 100): a precipitate is produced. Adjust the solution to a pH between 2.5 and 3.5 with dilute hydrochloric acid: the precipitate dissolves.

pH 2.5 ~ 3.5

Residue on Ignition Measure exactly a volume of Insulin Injection, equivalent to 500 to 1000 units according to the labeled units, in a tared platinum dish and evaporate slowly by heating on a water-bath to dryness. Add 2 drops of nitric acid to the residue and heat at first very gently, then strongly to incinerate. Place in a muffle furnace and heat at 600 ºC for 15 minutes, cool in a desiccator (silica gel) and weigh: the weight of the residue is not more than 1.0 mg for each labeled 1000 units.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 80 EU/100 insulin units.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement

Determination of Volume of Injection in Containers It meets the requirement

Nitrogen Content Perform the test as directed under the Nitrogen Determination: not less than 0.50 mg and not more than 0.64 mg of nitrogen (N: 14.01) is found for each labeled 100 units.

Assay Proceed with Insulin Injection as directed in the Assay under Insulin, and (5) Test solution and (9) Calculation are as follows.

(5) Test solution: According to the labeled units, dilute Insulin Injection with the diluent for Insulin to make two different test solutions, one to contain exactly 2.0 units in each mL which is designated as the high-dose test solution, T_H and the other to contain exactly 1.0 unit in each mL which is designated as the low-dose test solution, T_L.

(9) Calculation: Proceed as directed in the Assay under Insulin, using the following equation.

"Units in each mg of Insulin = antilog M x units in each mL of the high-dose standard solution $\times \frac{b}{a}$

a: Volume (mL) of the sample."

instead of following equation

Units in each mg of Insulin = antilog M × units in each

$$\text{mL of the high-dose standard solution} \times \frac{b}{a}$$

a: Mass (mg) of the sample

Containers and Storage ***Containers***—Hermetic containers.

Storage—In cold place, and avoid freezing.

Expiration Date 24 months after preparation.

Insulin Zinc Injection (Aqueous Suspension)

Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection. Insulin Zinc Injection (Aqueous Suspension) contains not less than 90.0 % and not more than 110.0 % of the labeled Insulin units and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.41) for each labeled 100 units.

Method of Preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. Each 100 mL of Insulin Zinc Injection (Aqueous Suspension) contains 0.15 to 0.17 g of sodium acetate trihydrate, 0.65 to 0.75 g of sodium chloride and 0.09 to 0.11 g of methyl parahydroxybenzoate.

Description Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and colorless, clear supernatant liquid and it readily becomes a suspension again on gentle shaking.
When it is examined microscopically, most part of the particles in the suspension are crystals, the dimension of which is mostly 10 to 40 μm. Others are amorphous with less than 2 μm.

Identification Adjust the pH of Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve and the solution is clear and colorless.

pH 7.1 ~ 7.5.

Purity ***Dissolved insulin***—Perform the test according to the Purity (2) of Insulin Zinc Protamine Injection (Aqueous Suspension).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 80 EU/100 insulin units.

Determination of Volume of Injection in Containers It meets the requirement.

Nitrogen content Perform the test as directed under the nitrogen determination: not less than 0.5 mg and not more than 0.64 mg of nitrogen (N: mol.wt. 14.01) is found for each labeled 100 Units.

Assay (1) ***Insulin***—Proceed as directed in the Assay under the Insulin Injection with the clear liquid obtained from Crystalline Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 100).

(2) ***Zinc***—Perform the test according to Assay (2) of Insulin Zinc Protamine Injection (Aqueous Suspension).

(3) ***Crystalline insulin***—Measure accurately Insulin Zinc Injection (Aqueous Suspension), equivalent to about 600 units according to the labeled units, centrifuge, discard the clear supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes and centrifuge. Discard the clear supernatant liquid and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 55.0 and not more than 70.0 % of the total nitrogen content. Calculate the total nitrogen content for insulin units of a sample from the values of nitrogen obtained in the Nitrogen content.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In cold place, and avoid freezing.

Expiration Date 24 months after preparation.

Iodine

I: 126.90

Iodine contains not less than 99.5 % and not more than 101.0 % of iodine (I).

Description Iodine appears as grayish black plate or heavy, granular crystals and has a metallic luster and a characteristic odor.
Iodine is freely soluble in ether, soluble in ethanol (95), sparingly soluble in chloroform and very slightly soluble in water.
Iodine dissolves in potassium iodide TS.
Iodine sublimes at room temperature.

Identification (1) A solution of Iodine in ethanol (95) (1 in 50) shows a red-brown color.

(2) A solution of Iodine in chloroform (1 in 1000) shows a red-purple to purple color.

(3) Add 0.5 mL of starch TS to 10 mL of a saturated solution of Iodine: a dark blue color is observed. When the mixture is boiled, the color disappears and it reappears on cooling.

Purity (1) ***Non-volatile residue***—Sublime 2.0 g of Iodine in a water-bath and dry the residue at 105°C for 1 hour: the weight of the residue is not more than 1.0 mg.

(2) ***Chloride or bromide***—Mix 1.0 g of finely powdered iodine with 20 mL of water and filter the mixture. To 10 mL of the filtrate, add diluted sulfurous acid (1 in 5) drop-wise until the yellow color disappears. Add 1 mL of ammonia TS, followed by 1 mL of silver nitrate TS in small portions and add water to make 20 mL. Shake well, filter and after discarding the first 2 mL of the filtrate, take 10 mL of the subsequent filtrate. To the filtrate, add 2.0 mL of nitric acid and water to make 20 mL: the solution so obtained has no more turbidity than the following control solution.

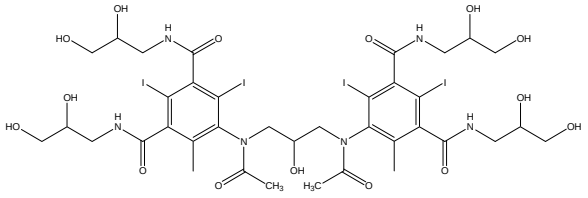
Control solution—To 0.20 mL of 10 mmol/L hydrochloric acid VS, add 5 mL of water, 2.5 mL of ammonia TS, 1 mL of silver nitrate TS, 2.0 mL of nitric acid and water to make 20 mL.

Assay Place 1 g of potassium iodide and 1 mL of water in a glass-stoppered flask, weigh accurately, add 0.3 g of Iodine to the flask and weigh accurately again. Dissolve the iodine by gentle shaking, add 20 mL of water and 1 mL of dilute hydrochloric acid and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.690 mg of I

Containers and Storage ***Containers***—Tight containers.

Iodixanol



$C_{35}H_{44}I_6N_6O_{15}$: 1550.18

5-{*N*-[3-(*N*-{3,5-*bis*[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl] acetamido}-1-*N*,3-*N*-*bis*(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide [*92339-11-2*]

Iodixanol contains not less than 98.6 % and not more than 101.0 % of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$), calculated on the anhydrous basis.

Description Iodixanol is a white, amorphous powder and is odorless.
Iodixanol is very soluble in water.
Iodixanol is hygroscopic.

Identification (1) Determine the infrared spectra of Iodixanol and Iodixanol RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention times of the two principal peaks obtained from the test solution of the related substance (2) corresponds to these obtained from the standard solution (2) in the Purity. A third isomer may appear as a minor peak.

(3) Take about 0.5 g of Iodixanol, in the crucible, heat: purple vapors are evolved.

Specific Optical Rotation $[\alpha]_D^{25}$: -0.5 ~ +0.5° (1 g, water, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Iodixanol according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Free iodine***—Weigh 2.0 g of Iodixanol to a glass-stoppered tube, add 20 mL of water, 5 mL of toluene, and 5 mL of 1mol/L sulfuric acid TS, shake vigorously, and allow to stand: the toluene layer shows no red or pink color.

(3) ***Free iodide***— Weigh 5.0 g of Iodixanol, add about 30 mL of water, dissolve and titrate with 0.001mol/L silver nitrate VS. (potentiometric titration, Endpoint Detection Method in Titrimetry). Not more than 10 μg of iodide per g is found.

Each mL of 0.001N silver nitrate VS
= 126.9 μg of I.

(4) ***Free aromatic amine***— Weigh accurately about 0.2 g of Iodixanol, dissolve in 15 mL of water, and use this solution as the test solution. Dissolve an accurately weighed quantity of Iohexol Related Substance III RS in water, and prepare a solution containing 10 μg per mL, Add 5 mL of water to 10.0 mL of this solution, and use this solution as the standard solution. Separately, use 15 mL of water as the blank solution. Place the standard solution, the test solution, and the blank solution, in an ice bath for 5 minutes. Add 1.5 mL each of 6 mol/L hydrochloric acid, mix by swirling, add 1.0 mL each of sodium nitrite solution (2 in 100), mix, and allow to stand in the ice bath for 4 minutes. Remove each solution from the ice bath, add 1.0 mL of 4 % sulfamic acid solution, and swirl gently until gas evolution ceases. Add 1.0 mL of *N*-(1-Naphthyl) ethylenediamine dihydrochloride solution, a freshly prepared solution of that in a mixture of propylene goycol and water (70 : 30) mix, add water to make exactly 25 mL, and allow to stand for 5 minutes. And compare the color: the color obtained from the test solution is lighter than the color obtained from the standard solution (not more than 0.05 %). If the color obtained from the test solution is about the same color

or darker than the color obtained from the standard solution, concomitantly determine the absorbances of the solutions obtained from the blank solution, the test solution and the standard solution, A_B, A_T and A_S, as directed under Ultraviolet-visible Spectrophotometry at the wavelength of maximum absorption of about 495 nm using the water as blank and 5-cm cells in length (not more than 0.05 %).

$$\text{Amount (\%) of free aromatic amine} = \frac{C}{W} \times \frac{A_T - A_B}{A_S - A_B}$$

C: Concentration (μg /mL) of Iohexol Related Compound III RS in the standard solution.
W: Amount (mg) of Iodixanol taken.

(5) ***Calcium***—Weigh accurately about 2.0 g of Iodixanol, dissolve in 10 mL of water, add 2 mL of internal standard solution, add water to make exactly 20 mL, and use this solution as the test solution. Perpare a solution containing 10 μg of Calcium per 1 mL with the standard Calcium solution, pipet 0.5, 2.5, 5.0, and 10.0 mL of this solution, add 5.0 mL each of the Internal standard solution, add water to make exactly 50 mL, and use these solutions as the standard solution (1), (2), (3) and (4). Pipet 5.0 mL of the internal standard solution, add water to make 50 mL, and use this solution as the blank test solution. Perform the test with the test solution and the standard solution (1), (2), (3) and (4) as directed under Atomic absorption spectrophotometry. Concomitantly determine the absorbances of each *s*tandard solution and the test solution at 393.37 nm, the calcium emission line, and at 361.38 nm, the scandium emission line, using the blank solution as the blank. Prepare a calibration line of the ratio of the calcium absorption to the scandium absorption versus the respective calcium concentrations. From the calibration line so obtained, determine the calcium concentration, *C*, in μg per mL, in the test solution. Calculate the content of calcium, in μg per g (not more than 5 μg per g):

$$\text{Amount (μg /g) of calcium} = 20 \times \frac{C}{W}$$

W: Amount (g) of Iodixanol taken to prepare the test solution.

Internal standard solution—Accurately weigh about 3.067g of scandium oxide, and dissolve in 1000 mL of water, add water to make 1000 mL.

(6) ***Ionic substances***—Use all gloass upparatus washed with water. Determine the specific conductance of a solution of Iodixanol (2 in 100), the conductance of a solution of ionic substancd is not more than a solution of Sodium Cholride (4 μg per mL) (not more than 0.02 %).

(7) ***Methanol, 2-propanol, and methoxyethanol***—Transfer about 0.25 g of Iodixanol, accurately weighed, to a headspace vial. Add 1.0 mL of the Internal standard solution, seal the vial, mix until dissolved, and use this solution as the test solution. Weigh accurately about 0.5 g of methanol, about 1.0 g each of 2-propanol and methoxyethanol, add water to make exactly 500 mL. Add water to 5.0 mL of this solution to make exactly 100 mL. Pipet 10.0 mL of this solution and 1.0 mL of internal standard solution, add water to make exactly 100 mL. Pipet 1.0 mL of this solution, seal, and use this solution as the standard solution. This solution contains about 0.005 mg of methanol, 0.01 mg each of 2-propanol and methoxyethanol. Perform the test with 1 mL each of test solution and standard solution as directed under Gas Chromatography according to the following operating conditions. Calculate the ratio of each peak area of methanol, 2-propanol and methoxyethanol to the peak area of the internal standard, Q_T and Q_S, respectively. Amount of methanol, 2-propanol and methoxyethanol per1 g of Iodixanol are not more than 50 μg, respectively.

$$\text{Amount (\%) of methanol, isopropanol and methoxyethanol} = 100 \times \frac{C}{W} \times \frac{Q_T}{Q_S}$$

C : Concentration (mg/mL) of each related substance.
W : Amount (mg) of Iodixanol taken.

Internal standard stock solution—Weigh about 0.5 g of secondary butyl alcohol, add water to make 500-mL, and add water to 1.0 mL of this solution to make 100 mL.

Operating conditions

Detector: A hydrogen flame ionization detector (FID)

Column: A capillaray column about 0.54 mm in internal diameter and about 30 m in length, coated with 1 μm thickness of polyehtyleneglycol compound (carbowax 20M).

Column temperature: Maintain at 40 ºC for 3 minutes, then it is increased linearly at a rate of 8 ºC per minute to 100 ºC, and is maintained at 100 ºC for 1 minute.

Injection port temperature: 150 ºC
Detector temperature: 200 ºC
Carrier gas: Helium
Flow rate: 11 mL/minute
System suitability

System performance: When the procedure is run with 1 mL of the standard solution according to the above operating conditions, methanol, 2-propanol, 2-butanol and methoxy ethanol are eluted in this order, with the resolution between methanol peak and 2-propanol peak being not less than 1.0.

System repeatability: When the test is repeated 6 times with 1 mL each of the standard solution under the above operating conditions, the relative standard

deviation of the ratios of the peak area for methanol and for 2-propanol is not more than 0.5 %, and the relative standard deviation of the ratio of the peak area for methoxy ethanol is not more than 10 %.

(8) ***Related substances***—(i) Weigh exactly an amount of Iodixanol, equivalent to about 0.5 g of anhydrous Iodixanol, dissolve in water make exactly 20 mL, use this solution as the test solution (1). Add water to 5.0 mL of the test solution (1) make esactly 50 mL, and use this solution as the test solution (2). Separately, quantitatively, dissolve accurately weighed quantity of Iodixanol RS, in water to obtain a solution having a know concentration of about 12.5 mg of anhydrous Iodixanol per mL, and use this solution as standard stock solution (1). Quantitatively dissolve an accurately weighed quantity of Iodixanol related substances I RS in water to obtain a solution having a known concentration of about 0.25 mg of anhydrous iodixanol related substance I per mL, and use this solution as the standard stock solution (2). Quantitatively dissolve an accurately weighed quantity of Iodixanol related substance II RS in water to obtain a solution having a known concentration of about 0.025 mg of anhydrous iodixanol related substance II per mL, and use this solution as the standard stock solution (3). Pipet 2.0 mL of the standard stock solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5.0 mL of the standard stock solution (1), 2.5 mL of the standard stock solution (2) and 2.5 mL of the standard stock solition (3), add water to make exactly 25 mL, use this solution as the standard solution (2).

Pipet 5.0 mL of the test solution (1) and 2.5 mL of the standard stock solution (2), add water to make exactly 50 mL, and use this solution as the control solution.

Use water as the blank solution. Perform the test with 10 μL each of the blank solution, the test solution (1) and the test solution (2) as directed under Liquid Chromatography according to the following operating conditions. When it is specified to proceed as directed for High-low chromatography obtained from test solution (1) and test solution (2), calculate the amount (%) of each related substance according to the following formula.

$$\text{Amount (\%) of related substance} = \frac{10X}{0.1Y+Z} \qquad (1)$$

X : Peak area of the specified related substance obtained from the test solution (1).

Y : Total area of all the peaks eluted before and after Iodixanol peak obtained from test solution (1), disgarding any peaks due to injection noise or solvent.

Z : Sum of peak area of the peak of all related substances and the principal peak of Iodixanol obtained from test solution (2).

① Iohexol—If Iohexol is present, it exhibits two peaks with retention times of about 0.37 and 0.39 relative to the principal iodixanol peak, in the chromatogram obtained from the test solution (1). Draw a baseline at the height of the baseline obtained from the blank solution. Calculate the total area of the two peaks and the amount of iohexol according to the equation(1) (not more than 0.6 %).

② Iodixanol related substance III—If iodixanol related substance III is present, it elutes as a single peak with a retention time of about 0.34 relative to the principal iodixanol peak, in the chromatogram obtained from test solution (1). Determine the peak area of Iodixanol revlated substaned III and calculate the amount of Iodixanol related substance III according to the equation(1) (not more than 0.2 %).

③ Iodixanol related substance I—If iodixanol related substance I is present, only the first and larger peak, with a retention time of about 1.07 relative to the principal iodixanol peak, is seen between the two principal iodixanol peaks in the chromatogram obtained from test solution (1); the second iodixanol related substance I peak co-elutes with iodixanol. The area of the first is about 80 % of the total area of related substance I, determine the area of the first peak, X_2. Calculate the amount of related substance I by formula (not more than 0.4 %).

$$\text{Amount (\%) of Iodixanol related substance I} = \frac{12.5X_2}{0.1Y+Z} \qquad (2)$$

Y and Z are: See formula (1).

④ Iodixanol related substance IV—If iodixanol related compound IV is present, only the first peak with a retention time of about 0.8 relative to the principal iodixanol peak, can be seen in the chromatogram obtained from test solution (1); the second peak co-elutes with Iodixanol. The area of the first peak corresponds to about 25 % of the total area of Iodixanol related substance IV. Determine the area of the first peak, calculate the amount of iodixanol related substance IV by following formula (not more than 0.2 %).

$$\text{Amount (\%) of Iodixanol related substance IV} = \frac{40X_1}{0.1Y+Z} \qquad (3)$$

Y and Z are: See formula (1).

⑤ Iodixanol related substance V— If iodixanol related compound V is present, the second peak only, with a retention time of about 1.18 relative to the last iodixanol peak, is seen in the chromatogram obtained from test solution (1); the first peak co-elutes with iodixanol. The area of the second peak corresponds to about 85 % of the total area of iodixanol related substance V, determine the area of the second peak, X3, calculate the amount of the related substance V by the following formula (not more than 0.2 %).

$$\text{Amount (\%) of Iodixanol related substance V} = \frac{10X_3}{0.85(0.1Y + Z)} \quad (4)$$

Y and Z are: See formula(1).

⑥ Overalkylated related substances—These compounds exhibit after iodixanol related compound V, with a retention time greater than 1.18 relative to the last iodixanol peak in the chromatogram of the test solution(1). Calculate the amount of overalkylated related substances by the formula (1) determining the amount of the related substances (not more than 1.0 %).

⑦ Unspecified related substance 1—Determine the peak area other than the above peak among the peak be shown before or after iodixanol prineipal peak in the chromatogram obtained from test solution (1), calculate the amount of unspecified related substance 1 according to the formula (1).

⑧ Other unspecified related substance 2—Determine the peak area other than the above peak among the peak be shown between principal peaks of iodixanol in the chromatogram obtained from the test solution (1), calculate the amount of other unspecified related substance 2. Amounts of each related substances of ⑦ and ⑧ are not more than 2.0 %, and amount of total unspecified related substances is not more than 0.5 %.

⑨ Total related substances—Determine total area of all peaks shown between the principal peaks of iodixanol in the chromatogram obtained from the test solution (1), calculate the amount of total related substance (not more than 1.5 %).

$$\text{Amount (\%) of total related substances} = \frac{\left[100\left(Y - X_1 - X_3 + \frac{X_1}{0.25} + \frac{X_2}{0.8} + \frac{X_3}{0.85}\right)\right]}{10(0.1Y + Z)}$$

in which the variables are as defined above.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μm in particle diameter).

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of acetonitrile and water (1 : 1)

Mobile phase B: water

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	6	94
0-30	6→20	94→80
30-70	20→100	80→0
70-80	100	0
80-81	100→6	0→94
81-90	6	94

Flow rate: 1.0 mL per minute

System suitability

Chromatograph the solutions asdirected for procedure, in the following injection sequence : Blank solution, standard solution(1), control solution and at least three replicates of standard solution(2).

System performance: When the procedure is run with 10 μL of the standard solution (1) according to the above operating conditions, the chromatogram obtained from the standard solution (1) exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are about 60 % and 40 %. If the chromatogram exhibits three principal peaks, their relative areas are about 60 %, 38 %, and 2 %. When the procedure is run with 10 μL of the standard solution (2) according to the above operating conditions, the chromatogram obtained from standard solution (2) exhibits two resolved peaks due to Iodixanol related substance II that elute before the Iodixanol peaks and one Iodixanol related substance I peak between the two principal Iodixanol peaks. The area of the two Iodixanol related substance II peaks is between 0.075 % and 0.125 % of the total area. Disregard any peak due to the solvent front and any peak corresponding to those obtained from the blank solution.

System repeatability : When the test is repeated 3 times with 10 μL each of the standard solution (2) according to the above operating conditions, the relative standard deviation of sum of areas of two isomer peaks obtained from Iodixanol related substance II is not more than 5 %. Measure the height of the Iodixanol related substance I peak, and adjust the sensitivity of the amplfier to obtain a peak height between 80 % and 100 % of the full scale. Measure the height, A, above the baseline of the Iodixanol related substance I peak and the height, B, above the baseline of the lowest part of the curve separating this peak from the first principal Iodixanol peak: A is not less than 1.3B. Perform the test with 10 μL of the control solution according to the above operating conditions: the peak of Iodixanol related substance I measurable.

(ii) Dissolve an accurately weighed quantity of Iodixanol, equivalent to about 0.125 g of anhydrous Iodixanol, in water to make exactly 50 mL, and use this solution as the test solution. Quantitatively dissolve an accurately weighed quantity of Iodixanol RS, equivalent to about 0.125 g of anhydrous Iodixanol, in water to make 10 mL, and use this solution as the standard stock solution (1). Weigh accurately quantity of Iodixanol related substance II RS, equivqlent to about 0.125 g of anhydrous Iodixanol, dissolve in water to

make exactly 100 mL, add water to 2.0 mL of this solution to make exactly 100 mL, and use this solution as the standard stock solution (2). Quantitatively dissolve an accurately weighed about 25 mg of Iodixanol related substance VI RS in water to make exactly 10 mL, and use this solution as the standard solution (3). Pipet 2.0 mL of standard stock solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5.0 mL of the standard stock solution (1) and 2.5 mL of the standard stock solution (2), add water to make exactly 25 mL, and use this solution as the standard solution (2). Pipet 1.0 mL of standard solution (1) and 1.0 mL of standard stock solution (3), add water to make 3.0 mL, and use this solution as the standard solution (3). Use water as the blank solution. Perform the test with 10 μL each of the test solution, the standard solution (1), the standard solution (2) and the standard solution (3) as directed under Liquid Chromatography according to the following operating conditions. Inject 3 times repeatly for the standard solution (2). Compare the retention times of the peaks obtained from the standard solution (3) to those obtained from the test solution. Iodixanol related substance VI exhibits two peaks, the second of which may partly overlap with another peak, and use only the area of the first and larger peak, corresponding to about 60 % of the total area of Iodixanol related substance VI. Draw baseline at the height of the baseline obtained from the blank solution. Calculate the area percent of Iodixanol related substance VI, {5-[[3-[[3-[[2,3-dihydroxypropyl)amino] carbonyl]-5-[[amino]carbonyl]-2,4,6-triiodphenyl] (acetylimino]-2-hydroxypropyl]-(acetylimino)]-*N,N′-bis*(2,3-dihydroxypropyl)-2,4,6-triiod-1,3-benzenedicar boxide}, by dividing the area obtained from the test solution 0.6 is not more than 0.3 %. Calculate the amount of the related substance VII shown as a single peak, with a shoulder, on the tail of the Iodixanol peak is not more than 0.6 %.

Operating conditions

Detector: An ultraviolet absorption photometry (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with monomolecular layer aminopropylsilanized silica gel for the liquid chromatography (5 μm particle diameter).

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Acetonitrile

Mobile phase B: Water

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	85	15
0-25	85→66	15→34

Flow rate: 2.5 mL per minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (1) according to the above operating conditions: the chromatogram obtained from standard solution (1) exhibits three principal unresolved peaks,the relative peak areas are about 62 %, 35 % and 3 %; and the retention time of the last Iodixanol peak is not more than 14 minutes. When the procedure is run 10 μL of the standard solution (2) according to the above operating conditions: the chromatogram obtained from standard solution (2) exhibits two partially unresolved peaks due to Iodixanol related substance II with relative retention times of about 0.33 and 0.39, the peak area of Iodixanol related substance II is between 0.075 % and 0.125 % of the total area. When the procedure is run with 10 μL of the standard solution (3) according to the above operating conditions: the chromatogram obtained from standard solution (3) exhibits two unresolved peaks due to iodixanol related substance VI, with relative retention times of about 0.67 and 0.72. The resolution, between the first peak of iodixanol related substance VI and the first principal peak of Iodixanol is not less than 5.0.

System repeatability: When the test is repeated 3 times with 10 μL each of standard solution (2) according to the above conditions: the relative standard deviation of total peak areas of two isomers of the related substance II is not more than 5 %.

Water Not more than 4.0 % (0.5 g, volumetric titration, direct titration).

Microbial Limit The total aerobic microbial count is not more than 100 CFU/g.

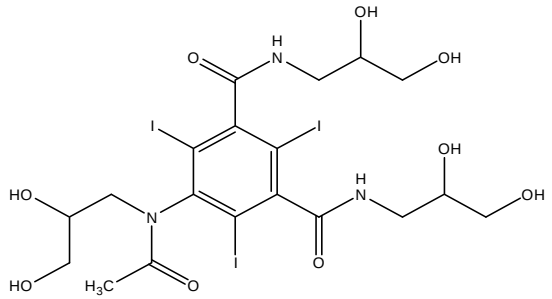
Assay Weigh accurately about 0.5 g of Iodixanol, add 25 mL of a sodium hydroxide solution (1 in 20) and 0.5 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 1 hour. Cool the flask to room temperature, and wash the condenser with 20 mL of water, combine the washing with the reaction mixture, filter. Wash the flask and the filter with several small portions of water, filter, and add the filtered washings to the filterate. Add 5 mL of acetic acid (100), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 N silver nitrate
= 25.84 mg of $C_{35}H_{44}I_6N_6O_{15}$.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Iohexol



$C_{19}H_{26}I_3N_3O_9$: 821.14

1-*N*,3-*N*-*bis*(2,3-Dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodobenzene-1,3-dicarboxamide [*66108-95-0*]

Iohexol contains not less than 98.0 % and not more than 102.0 % of iohexol ($C_{19}H_{26}I_3N_3O_9$), calculated on the anhydrous basis.

Description Iohexol is a white or off-white powder and is odorless.
Iohexol is freely soluble in water or in methanol, and practically insoluble in ether or in chloroform.
Iohexol is hygroscopic.

Identification (1) Heat about 0.5 g in a crucible: purple vapors are evolved.

(2) Determine the absorption spectra of solutions of Iohexol and Iohexol RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit a maximum and a minimum of absorption at the same wavelengths.

(3) Determine the infrared spectra of Iohexol and Iohexol RS as directed in the potassium bromide disk method under Infrared Spectrometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Weigh 0.1 g each of Iohexol and Iohexol RS, dissolve in 10 mL of methanol, and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spots 10 μL each of the test solution and the standard solution a silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (50 : 25 : 11) to a distance of about 15 cm, and air-dry the plates. Examine under ultraviolet (254 nm): the presence of exo- and endo- isomers in the test solution and the standard solution is shown by the appearance of two spots, each of which correspond to the corresponding principal spot. The R_f values obtained from the test solution and the standard solution are the same.

Specific Optical Rotation $[\alpha]_D^{25}$: between -0.5 and +0.5 C (0.5 g, water 10 mL and 100 mL).

Purity (1) ***Clarity and color of solution***—Weigh 16.18 g of Iohexol, dissolve in water, to make exactly 25 mL, filter through a filter membrane having a porosity of 0.22 μm. Determine the absorbanas of this filterate as directed under Ultraviolet-visible Spectrophotometry and using water as the blank, determine the absorbances of this solution at 400, 420 and 450 nm are not more than than 0.180, 0.030, and 0.015, respectively.

(2) ***Heavy metals***—To 5.0 g of Iohexol add water to make 50 mL. Use 12 mL of this solution as the test solution. Separately, mix 10 mL of diluted standard lead solution with 2 mL of the test solution, and use this solution as the control solution. Separately, mix 10 mL of water with 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution, add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

Diluted standard lead solution—Immediately before use, pipet 5 mL of standard lead solution, and add water to make 50 mL.

System suitability: The control solution shows a slightly brown color compared to the blank solution.

(3) ***Free aromatic amine***— Transfer 0.2 g of Iohexol to a 50-mL volumetric flask, add 15 mL of water, and mix to dissolve. To a second 50-mL volumetric flask, add 5 mL of water and 10 mL of a solution containing 10 μg per mL by dissolving Iohexol related substance I {5-(amino)-*N*,*N*'-*bis*(2,3-dihydroxypropyl)-2,4,6-triiod-1,3-benzenedicarboxyamide)} RS in water. To a third 50-mL volumetric flask, add 15 mL of water. Place these solutions in an ice bath, and cool for 5 minutes. Add 3.0 mL each of 5 mol/L hydrochloric acid TS and swirl to mix. Add 2.0 mL of sodium nitrite solution (1 in 50) and allow to stand for 4 minutes. Add 2.0 mL each of sulfamic acd solution (1 in 25), shake, and allow to stand for 1 minute. Remove these solutions from the ice bath. To each flask, add 2 mL each, of a solution of *N*-(1-naphthyl)ehtylene-diamine dihydrochloride in dilute propylene glycol (7 in 10) (1 in 1000), mix, add water to make 50 mL, allow to stand for 5 minutes, and use these solutions as the test solution, the standard solution and the blank solution. Perform the test with these solutions as the directed under Ultraviolet-visible spectrophotometry: the absorbance of the test solution is not larger than the absorbance of the standard solution (not more than 0.05 %).

(4) ***Free iodine***—Transfer 2.1 g of Iohexol to a 50-mL centrifuge tube with a stopper, add about 20 mL of water, and shake vigorously to dissolve. Add 5 mL of

toluene and 5 mL of 1 mol/L sulfuric acid TS, shake, and centrifuge at high speed: the toluene layer shows no red or pink color.

(5) ***Free iodide***—Weigh 5.0 g of Iohexol, dissolve in about 20 mL of water, transfer to a centrifuge tube with a stopper, add 20 mL of water, and shake vigorously or heating weakly to dissolve, and titrate with 0.001 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction (0.001 %).

Each mL of 0.001 mol/L $AgNO_3$ = 126.9 μg of I

(6) ***Ionic substances***—Rinse all glassware 5 times with distilled water. Determine the specific resistance, (R_{SP}), at 20 ºC with a solution of Iohexol (1 in 50), calculate the specific conductance, k, taken by the formula: this specific conductance is not larger than the conductance of 0.0002 % sodium chloride (not more than 0.01 %).

$$\text{Specific conductance} = \frac{1}{R_{SP}} \times 106$$

(7) ***Methanol, isopropanol, methoxy ethanol***—Transfer about 6.25 g of Iohexenol exactly weighed, add 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the test solution (1). Transfer 5.0 mL of the test solution (1) and 1 mL of water to a vial with gum stopper. Heat the sealed vial at 95 ºC for 15 minutes, use this solution as the test solution (2). Transfer 5.0 mL of the test solution (1) and 1 mL of s the tandard solution (2) to a vial with gum stopper. Heat the sealed vial at 95 ºC for 15 minutes, and use this solution as the test solution (3). Transfer 5.0 mL of t the test solution (1) and 1.0 mL of the standard solution (3) to a vial with gum stopper. Heat the sealed vial at 95 ºC for 15 minutes, and use this solution as the test solution (4). Transfer 5.0 mL of the test solution (1) and 1.0 mL of the standard solution (4) to a vial with gum stopper. Heat the sealed vial at 95 ºC for 15 minutes, and use this solution as the test solution (5). Separately, weigh accurately about 0.6 g of methanol, add about 100 mL of water, mix, add about 0.6 g of 2-propanol, accurately weighed, add about 100 mL, of water, and mix. To this solution add about 0.6 g of methoxy ethanol (95), accurately weighed, add 100 mL of water, mix, add water to make exactly 1000 mL, and use this solution as the standard solution (1). Pipet 10.0 mL of the standard solution (1), add water to make exactly 50 mL, pipet 10.0 mL of this solution, add water to make 100 mL, and use this solution as the standard solution (2). Pipet about 5.0 mL of standard solution (1), add water to make exactly 100 mL, and use this solution as the standard solution (3). Add water in 10.0 mL of standard solution (1) to make exactly 100 mL, and use this solution as the standard solution (4). Add water in 10.0 mL of the standard solution (4) and 10.0 mL of the internal standard solution to make exactly 50 mL, transfer 6.0 mL of this solution to a vial fitted with a septum and crimp cap, and seal, heat the sealed vial at 95 ºC for 15 minutes, and use this solution as the standard solution (5). Perform the test with 2 mL each of the test solution (2), the test solution (3), the test solution (4), the test solution (5) and the standard solution (5) as directed under Gas Chromatography according to the following operating conditions using the injection port apparatus for the headspace, calculate the peak areas of the principal peak from each chromatograms. Plot the peak area ratios obtained from the test solutions against the quatity of amount of methanol, amount of 2-propanol and amount of methoxyethanol added per g of Iohexol. Extrapolate the line joining the points until it intercepts the concentration axis. The distance between this point and the intersection of the axis is the amount (mg/g) of methanol, 2-propanol and methoxyethanol. Amounts (mg/g) of methanol and 2-propanol are not more than 0.005 %, respectively, the amount of methoxyethanol is not more than 0.002 %.

Internal standard solution−Prepare a solution of 2-butanol in water containing about 0.05 mg per each mL.

Operating conditions

Detector : A hydrogen flame-ionization detector

Column : A fused silica capillary column, about 0.53 mm in internal diameter and 30 m in length, the inside coated with a 3-μm layer of 6 % cyanopropylphenyl and 94 % dimethylpolysiloxane.

Column temperature : It is held at 40 ºC for 5 minutes and is programmed to increase at a rate of 10 ºC per minute to 100 ºC, maintain at 100 ºC for 1 minute.

Inject port temperature : 140 ºC

Detector temperature : 250 ºC

Carrier gas : helium

Flow rate : 14 mL/minute

System suitability

System performance : When the procedure is run with standard solution (5) according to the above operating conditions, the relative retention times of methanol, 2-propanol, 2-butanol and methoxy ethanol are about 0.3, 0.5, 1.0 and 1.3, respectively, with the resolution between methanol peak and 2-propanol peak being not less than 2.5.

System repeatability: When the test is repeated 6 times with standard solution (5) according to the above operating conditions, the relative standard deviation of each peak area is not more than 5 %.

(8) ***3-chloro-1,2-propanediol***— Dissolve about 1 g of Iohexol, accurately weighed, in 1.0 mL of water, extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to the volume of 2.0 mL, using a warm water bath and a stream of nitrogen.

Pass this solution through a membrane filter, and use the clear filtrate as the the test solution. Separatry, dissolve an accurately weighed quantity of 3-chloro-1,2-propanediol quantitatively in ethyl acetate to obtain the solution having a known concentration of about 20 μg per mL, and use this solution as the standard solution. Perform the test with about 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions: 3-chloro-1,2-propanediol, a mixture of two isomers, exhibits two principal peaks at 12 and 12.5 minutes, respectively. Measure the sums of two peak areas obtained from 3-chloro-1,2-propanediol, A_{ST} and A_{SA}, calculate the amount of 3-chloro-1,2-propanediol (not more than 0.0025 %).

$$\text{Amount (μg) of 3-chloro-1,2-propanediol} = 100 \times \frac{A_{ST}}{A_{SA}} \times \frac{2C_{ST}}{R}$$

C_{ST}: Concentration (μg/mL) of 3-chloro-1,2-propanediol in the standard solution.

R: The recovery ratio (%) measured in the system suitability test.

Operating conditions

Detector : A hydrogen flame-ionization detector.

Column : A fused silica capillary column, about 0.32 mm in internal diameter and 30 m in length, the inside coated with a 1-μm layer of 14 % cyanopropylphenyl-86 % methylpolysiloxane.

Column temperature : At first, allow to stand at 50 ºC for 2 minutes and is programmed to increase at a rate of 10 ºC per minute to 200 ºC.

Injection port temperature : About 230 ºC

Detector temperature : About 250 ºC

Carrier gas : helium

System suitability

System performance: When the procedure is run with the system suitability solution according to the above operating conditions, the recovery rate of 3-chloro-1,2-propanediol obtained from iohexanol is between 60 and 90 %.

$$\text{Recovery rate (%) of 3-chloro-1,2-propanediol} = 100 \times \frac{A_{RC}}{A_{ST}} \times \frac{C_{ST}}{C_{RS}}$$

C_{RS}: Concentration (μg/mL) of 3-chloro-1,2-propanediol obtained from the system suitability solution.

C_{ST}: Concentration (μg/mL) of 3-chloro-1,2-propanediol obtained from the standard solution.

A_{RC}: Peak area of 3-chloro-1,2-propanediol obtained from the system suitability solution.

A_{ST}: Peak area of 3-chloro-1,2-propanediol obtained from the standard solution.

System repeatability: When the test is repeated 6 times with the standard solution according to the above condotions: the relative standard deviation of peak area is not more than 10 %.

System suitability solution: Dissolve 1 g of Iohexol containing less than 5 μg of chloropropanediol in 1 mL of water. Quantitatively dissolve an accurately weighed quantity of 3-chloro-1,2-propanediol in ethyl acetate to obtain a solution having a concentration of about 25 μg per mL, Add 2.0 mL of ethyl acet ate solution to the aqueous solution of Iohexol in a separator, amd mix. Transfer the ethyl acetate layer to a separate containers, and extract the aqueous layer three additional times with 2 mL of ethyl acetate, combining all four extracts. Dry the combined extracts using anhydrous sodium sulfate, filter, and wash the filter with a small amount of ethyl acetate. combine the wash, concentrate to 2.0 mL under nitrgen stream, filter with membrane filter, and use the filterate as the system suitability solution.

(9) ***Related substances*—**Weigh accurately 75 mg of Iohexol, add water, dissolve to make 50 mL, and use this solution as the test solution. Perform the test with 10 μL of this solution as directed under Liquid Chromatography according to the following operating conditions, and determine the amount of each the related substances: the peaks other than the principal peak are not more than 0.1 %, respectively, the peaks of *O*-alkylated substances are not more than 0.6 %, the sum of all the related substances other than *O*-alkylated substances is not more than 0.3 %.

$$\text{Amount (%) of the related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Each peak area other than the principal peak

A_S: Sum of the area of all peaks

Operating conditions

Detector : An ultraviolet absorption Photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle size diameter).

Mobile phase : Change the rate of acetonitrile and water, the chromatograph is programmed to provide variable mixtures of these solvents, the percentage of acetonitrile increases from 1 % to 13 % at an increase rate of 0.2 % per minute.

Flow rate : 1.0 mL/minute

System suitability

System performance : When the procedure is run with 10 μL of system suitability solution according to the above conditions, the relative retention time for the *O*-alkylated substances is between 1.1 and 1.4 to 1.0 of the retention time for the *exo*-isomer of iohexol with the resolution between iohexol related substance II and iohexol related substance III being not less than 20.0, and the peak a rea of iohexol related substance III is

0.5 % ± 0.1 % by comparison to the total area of all of the peaks in the chromatogram.

System suitability solution—Dissolve acculately weighed quantities of Iohexol RS, Iohexol related substance I RS, and Iohexol related substance III RS in water to obtain a solution having known concentrations of about 1.5, 0.0075 and 0.0069 mg per mL, repectively, and use this solution as the system suitability solution.

Water Not more than 4.0 % (0.3 g, volumetric titration, direct titration).

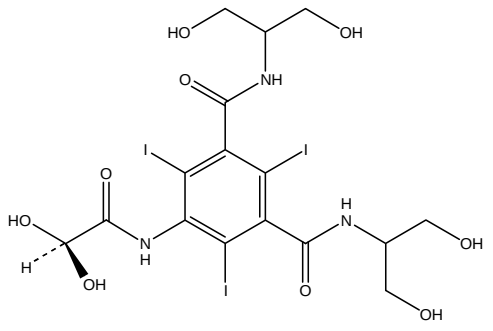
Assay Weigh accurately about 0.5 g of Iohexol, add 25 mL of 1.25 mol/L sodium hydroxide TS and 0.5 g of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, add the washing in the reaction mixture, mix, and filter the mixture. Rinse the flask and the filter throughly with small portions of water, sum the rinsings to the filtrate. Add 5 mL of acetic acid (100), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L silver nitrate
= 27.37 mg of $C_{19}H_{26}I_3N_3O_9$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Iopamidol



$C_{17}H_{22}I_3N_3O_8$: 777.09

1-*N*,3-*N*-*bis*(1,3-Dihydroxypropan-2-yl)-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1,3-dicarboxamide [*60208-45-9*]

Iopamidol, when dried, contains not less than 99.0 % and not more the 101.0 % of iopamidol ($C_{17}H_{22}I_3N_3O_8$).

Description Iopamidol is a white, crystalline powder. Iopamidol is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identifiation (1) To 0.05 g of Iopamidol add 5 mL of hydrochloric acid, heat for 10 minutes in a water bath: the test solution responds to the Qualitative Tests for primary aromatic amines.

(2) Heat 0.1 g of Iopamidol over a flame: a purple gas is evolved.

(3) Determine the infrared spectra of Iopamidol and Iopamidol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_{436\ \mathrm{nm}}^{20}$: –4.6 ~ –5.2º (after drying, 4 g, water, warm, after cooling, 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Iopamidol in 10 mL of water: the solution is clear and colorless.

(2) ***Free acid or alkali***—Weigh accurately about 10.0 g of Iopamidol, dissolve in 100 mL of freshly boiled and cooled water, and titrate with 0.01 mol/L hydrochloric acid VS or 0.01 mol/L sodium hydroxide VS until the pH of the test solution becomes 7.0: not more than 1.37 mL of 0.01 mol/L sodium hydroxide (not more than 0.005 % of free acid) or not more than 0.75 mL of 0.01 mol/L hydrochloric acid (not more than 0.003 % of free alkali) is consumed.

(3) ***Primary aromatic amines***—Dissolve 0.60 g of Iopamidol in 8 mL of water add 1 mL of a solution of sodium nitrite (1 in 50) and 12 mL of 2 mol / L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, allow to stand for 1 minute, and add 1 mL of naphthylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry using a solution, prepared in the same manner without Iopamidol, as the blank: the absorbance is not more than 0.12 (not more than 0.020 %).

(4) ***Iodine***—Dissolve 2.0 g of Iopamidol in 25 mL of water, add 5 mL of 1 mol/L sulfuric acid and 5 mL of toluene, shake well, and allow to stand:the toluene layer is colorless.

(5) ***Free iodine ion***—Weigh accurately about 5.0 g of Iopamidol, dissolve in 70 mL of water, and adjust the pH to about 4.5 with dilute acetic acid . To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titratewith 0.001 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Content of iodine ion in Iopamidol is not more than 0.001 %.

Each mL of 0.001 mol/L silver nitrate VS
= 0.1269 mg of I

(6) ***Heavy metals***—Moisten 1.0 g of Iopamidol with a small quantity of sulfuric acid, heat gradually to almost incinerate by a possibly lower temperature. After cooling, moisten again with a small quantity of sulfuric acid, heat gradually until white fumes no longer are evolved, and incinerate by ignition 450 to 550 °C. Proceed as directed in Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(7) ***Related substances***—Dissolve 0.10 g of Iopamidol in water to make exactly 10 mL, and use this solution as the test solution. Separately, dissolve 10 mg of *N,N'-bis*[2-hydroxy-1-(hydroxymethyl) ethyl]-5-hydroxyacetyl-amino–2,4,6-triiodoisophthalamide in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: each area of the peaks other than the peak of iopamidol from the test solution is not larger than the peak area of the standard solution, and the total of these areas is not larger than 2.5 times of the peak area of the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength; 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Water.

Mobile phase B: A mixture of water and methanol (3:1).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-6	92	8
6-18	92→65	8→35
18-30	65→8	35→92
30-34	8	92

Flow rate:Adjust the flow rate to 1.5 mL per minute.

System suitability

System performance: Dissolve 1 mL of the test solution and 10 mg of *N,N'-bis*[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodo-isophthalamide in water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N,N'-bis*[2-hydroxy-1-(hydroxy-methyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodo-isophthalamide and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N,N'-bis*[2-hydroxy-1-(hydroxy-methyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodo-isophthalamide is not more than 1.0 %.

Time span of measurement: About 4.3 times as long as the retention time of iopamidol.

Loss on Drying Not more than 0.3 % (1 g, 105°C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

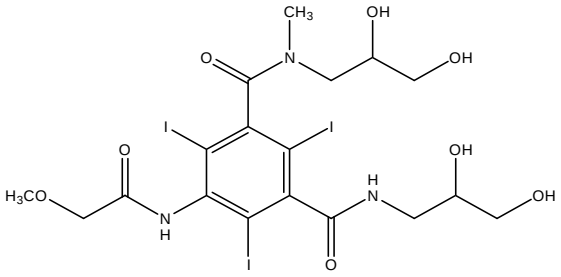
Assay Weigh accurately about 0.5 g of Iopamidol, previousiy dried, transfer to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, boil for 30 minutes under a reflux confenser, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washing with the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L silver nitrate VS
= 25.90 mg of $C_{17}H_{22}I_3N_3O_8$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Iopromide



$C_{18}H_{24}I_3N_3O_8$: 791.11

1-*N*,3-*N*-*bis*(2,3-Dihydroxypropyl)-2,4,6-triiodo-5-[(2-methoxyacetyl)amino]-3-*N*-methylbenzene-1,3-dicarboxamide [*73334-07-3*]

Iopromide contains not less than 97.0 % and not more than 102.5 % of iopromide ($C_{18}H_{24}I_3N_3O_8$), calculated on the anhydrous and solvent-free basis.

Description Iopromide appears as white or pale yellow powder.
Iopromide is freely soluble in water and in dimethylsulfoxide, and practically insoluble in acetone, in ethanol (95) or in ether.

Identification (1) Determine the infrared spectra of Iopromide and Iopromide RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Perform the test with Iopromide according to the related substances under the Purity: the principal sports obtained from the test solution and the standard solution show the same R_f values.

Purity (1) ***Heavy metals***—To 2.0 g of Iopromide add water to make 20 mL. Use 12 mL of this solution as the test solution. Separately, mix 10 mL of diluted standard lead solution with 2 mL of the test solution, and use this solution as the control solution. Separately, mix 10 mL of water with 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

Diluted standard lead solution—Immediately before use, pipet 5 mL of standard lead solution, and add water to make 50 mL.

System suitability: The control solution shows a slightly brown color compared to the blank solution.

(2) ***Free iodine***—Weigh 2.0 g of Iopromide, and dissolve in 20 mL of water. Add 2 mL of toluene and 2 mL of diluted surfuric acid, and shake vigorously: the toluene layer shows no red color.

(3) ***Free iodide***—Weigh 10.0 g of Iopromide, and dissolve in 70 mL of water. Adjust with 0.05 mol/L surfuric acid VS to a pH of 3.5 ± 0.5, titrate with 0.001 mol/L silve nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform the blank determination and make any necessary correction (not more than 0.002 %).

Each mL of 0.001 mol/L silver nitrate VS
= 126.9 μg of I.

(4) ***Free aromatic amine***—Weigh accurately 0.5 g of Iopromide, transfer to a 25-mL volumetric flask, add 20 mL of water to dissolve, and use this solution as the test solution. Separately, weigh accurately 25 mg of Iopromide Related Substance I [5-(acetylamino)-*N*,*N*'-*bis*(2,3-dihydroxypropyl-2,4,6-troiodo-N-methyl-1,3-benzene-dicarboxamide)] RS, and dissolve in water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 18.0 mL of water, and mix, and use this solution as the standard solution. Transfer 20 mL of water to a 25-mL volumetric flask, and use this solution as the blank solution. Place the flasks in an ice bath, and protect from light. Add slowly 1.0 mL of 8 mol/L hydrochloric acid TS, mix, and allow to stand for 5 minutes. Add 1.0 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 0.50 mL of freshly prepared sulfamic acid solution, (8 in 100). Shake each flask vigorously several times within the next 5 minutes, venting off the gas that evolves. Add 1.0 mL of freshly prepared solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30) (1 in 1000), and shake. Remove the flasks from the ice bath, and allow to stand in a water bath at about 25℃ for 10 minutes. Dilute with water to make exactly 25 mL, and degas with the aid of sonication for 1 minute. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at the wavelength maximum absorbance at about 495 nm using the blank solution. Calculate the percentage of aromatic amine in the portion of Iopromide taken by the following formula: it is not more than 0.10 %. The absorbance of the standard solution is not less than 0.40.

$$\text{Amount (\%) of aromatic amine} = 10 \times \frac{W_S}{W_U} \times \frac{A_U}{W_S}$$

W_S: Amount (mg) of Iopromide Related Substance I RS taken.
W_U: Amount (mg) of Iopromide taken.

(5) ***Ethanol***—Dissolve an exactly weighed 0.5 g of Iopromide in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately a suitable volume of ethanol (95), add *N,N*-dimethylformamide, mix, prepare a solution having a known concentration of about 0.050 mg of ethanol (C_2H_5OH) per mL, and use this solution as the standard solution. Use *N,N*-dimethylformamide as the blank solution. Transfer 2.0 mL each of the test solution, the standard solution and the blank solution to separate headspace vials, add 10 μL each of 1 mol/L hydrochloric acid TS to each vial, then seal the vials using a flanged cap. Perform the test as directed under Gas Chromatography according to the following operating conditions, and determine the peak area of ethanol, A_T and A_S (not more than 0.4 %).

$$\text{Concentration (mg/mL) of ethanol} = \frac{C}{I} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of ethanol in the standard solution.
I: Concentration (mg/mL) of iopromide in the test solution.

Operating conditions

Detector : A hydrogen flame ionization detector

Column : A capillary column about 0.25 mm in internal diameter and about 30 cm in length, the inside coated with a 1.4-μm layer of liquid phase 6 % cyanopropylphenyl-94 % dimethylpolysiloxane.

Column temperature : Programmed according to the following steps: it is held at 40 ºC for 10 minutes, then increased at a rate of 5 ºC per minute to 70 ºC; it is then increased at a rate of 30 ºC per minute to 220 ºC.

Inject port temperature : 160 ºC

Headspace sampler temperature : 80 ºC

Detector temperature : maintained at 250 ºC.

Carrier gas : helium

Flow rate : 27 mm/second

System suitability

System performance: When the procedure is run with 2.0 mL of the standard solution as directed under the above operating conditions, the retention time of ethanol is about 3 minutes.

System repeatability: When the test is repeated 3 times with 2.0 mL each of the standard solution, the relative standard deviation of the peak area is not more than 4.0 %. When the procedure is run with the blank solution according to the above operating conditions, the chromatogram shows no peak responses at the retention time for ethanol.

(6) ***N-acetyl compound (Iopromide related compound I)***—Using the chromatograms obtained in the Assay, calculate the percentage of *N*-acetyl compound in the Iopromide taken by the formula:

$$\text{Amount (\%) of N-acetyl compound} = 20\times\frac{W_B}{W_1}\times\left[\frac{(A_{Y1}+A_{Y2})}{(R_{Y1}+R_{Y2})}\right]$$

W_B: Amount (mg) of Iopromide related substance I RS taken to prepare the related substance I standard solution.

W_1: Amount (mg) of Iopromide taken to prepare the test preparation.

A_{Y1}, A_{Y2}: Peak areas of the Iopromide related substance I Y_1- and Y_2-isomers, respectively, in the chromatogram obtained from the test preparation.

R_{Y1}, R_{Y2}: Peak areas of the Iopromide related substance I Y_1- and Y_2-isomers, respectively, in the chromatogram obtained from the related substance I standard solution.

(7) ***Other related substances***—Dissolve about 0.1 g of Iopromide in a mixture of methanol and water (1:1) to make exactly 10 mL and use this solution as test solution. Separately, dissolve dilute appropriately a suitable amount of Iopromide RS in a mixture of methanol and water (1:1) to make exactly 0.01, 0.05, 0.1 and 0.2 mg per each mL, and use this solution as the standard solutions. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL and 2 μL of the test solution and 1 μL of each of the standard solutions on two separate plates of silica gel with fluorescent indicator for thin-layer chromatography. Place one plate in a development chamber containing the acidic eluant, and the second plate in a development chamber containing the basic eluant. Develop the plates to a distance about 15 cm, remove the plates from the chambers, and air-dry the plates. Both plates are exposed to ultraviolet light (main wavelength: 254 nm). Separately, the plate developed with the acidic eluant is exposed to ammonia vapors 10 to 30 minutes and air-dry the plates. Both plates are exposed to ultraviolet light until the principal spots appear yellow. Spray evenly with visusalization solution, and examine the plates under ambient light. Determine total concentration of all secondary spots, except those due to free aromatic amine and to the *N*-acetyl compound obtained in the test solution for limit of N-acetyl compound, is not more than 3.0 %.

Acidic eluant—chloroform : methanol : water : formic acid (62 : 32 : 6 : 2)

Basic eluant—1,4-dioxane : water : ethanol (95) (85 : 15 : 4)

Visualization solution—Dissolve 2.7 g of iron (III) chloride hexahydrate in a 100 mL of 2.4 mol/L hydrochloric acid, use this solution as the solution A. Store this solution in a refrigerator. Dissolve 3.5 g of potassium hexacyanoferrate (III) in 100 mL of water, use this solution as the solution B. Store this solution in a refrigerator. Dissolve 5.0 g of sodium arsenite in 30 mL of 1 mol/L sodium hydroxide solution that has been cooled to 0 ºC. While stirring mix with 65 mL of 2.4 mol/L hydrochloric acid, use this solution as the solution C, and store at room temparature. Use the clear supernatant. Mix 10 mL of solution A, 10 mL of solution B and 2.0 mL of solution C, and use this solution within 30 minutes after preparation.

(8) ***Isomer***—Using the chromatogram obtained from the test solution in the Assay, calculate the amounts of E_1- and Z_1-isomers of Iopromide according to the following equation formula, is between 49.0 and 60.0 %.

$$\text{Amounts (\%) of } E_1\text{- and } Z_1\text{-isomers of Iopromide} = 100(r_{B1}+r_{Z1})/(r_{B1}+r_{B2}+r_{Z1}+r_{Z2})$$

$$\text{Amounts (\%) of } E_2\text{- and } Z_2\text{-isomers of Iopromide} = 100(r_{E2}+r_{Z2})/(r_{E1}+r_{E2}+r_{Z1}+r_{Z2})$$

r_{E1}, r_{E2}, r_{Z1} and r_{Z2}: the peak areas of E_1-, E_2-, Z_1- and Z_2- isomers of Iopromide.

Water Not less than 1.5 % (1 g, volumetric titration method, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 38 mg of Iopromide and dissolve in a mixture of methanol and water (1 : 1) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 38 mg of Iopromide RS (previously measure the water), dissolve in a mixture of methanol and water (1 : 1), to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operatiing conditions and calculate the peak area of iopromide in each solutions. The test for the peak identification of E-isomer is run as follows. Add a constant volume of standard solution to a vial, seal, heat for 15 minutes at 121 ºC, and cool. Perform the test with the cooled solution according to the following operating conditions, and compare with the peak of standard solution unheated: identify with the retention time of two isomer peaks increasing the size after heating.

$$\text{Amount (mg) of iopromide } (C_{18}H_{24}I_3N_3O_8) = \text{Amount (mg) of Iopromide RS} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Iopromide RS in the standard solution.

A_T: Sum of peak area of E_1-, E_2-, Z_1- and Z_2-isomers obtained from the test solution.

A_S: Sum of peak area of E_1-, E_2-, Z_1- and Z_2-isomers obtained from the standard solution.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 254 nm)

Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase : Mix gently 6 g of chloroform with 59 g of methanol, then add 900 g of water, and do not stir or sparge the mobile phase during use. Allow the mobile phase to flow for not less than 60 minutes between each injection.

Flow rate : About 1.2 mL/minute

Column temperature: A constant temperature of about 20 ºC.

System suitability

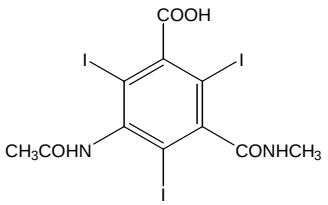
System performance: When the procedure is run with 10 μL of the standard solution as directed under the above operating conditions, the relative retention times for Iopromide E_1-isomer, iopromide E_2-isomer, iopromide Z_1-isomer, and iopromide Z_2-isomer are about 0.70, 0.75, 0.85, and 1.0, respectively with the resolution between iopromide isomers Z_1 and Z_2 being not less than 2.0; Separately, weigh accurately about 1.9 mg of Iopromide related substance I RS. Add the mixture of methanol and water (1 : 1), and dissolve to make exactly 100 mL.. When the procedure is run with 10 μL of this solution as directed under the above operating conditions, the relative retention times of Y_1- and Y_2-isomers of the iopromide related compound I are about 0.28 and 0.31, respectively, and the signal-to-noise ratio for Y_2-isomer peak is not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL each of standard solution according to the above operating conditions: the relative standard deviation of the peak areas of iopromide is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Iotalamic Acid



$C_{11}H_9I_3N_2O_4$: 613.91

3-Acetamido-2,4,6-triiodo-5-(methylcarbamoyl)benzoic acid [*2276-90-6*]

Iotalamic Acid, when dried, contains not less than 99.0 % and not more than 101.0 % of iotalamic acid ($C_{11}H_9I_3N_2O_4$).

Description Iotalamic Acid appears as white powder and is odorless.

Iotalamic Acid slightly soluble in ethanol (95), very slightly soluble in water and practically insoluble in ether.

Iotalamic Acid dissolves in sodium hydroxide TS.

Iotalamic Acid is gradually colored by light.

Identification (1) Heat 0.1 g of Iotalamic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared spectra of Iotalamic Acid and Iotalamic Acid RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of Iotalamic Acid in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Primary aromatic amines***—To 0.50 g of Iotalamic Acid, add 15 mL of water and dissolve in 1 mL of sodium hydroxide TS while ice-cooling. Add 4 mL of a solution of sodium nitrite (1 in 100) to the solution, immediately add 12 mL of 1 mol/L hydrochlo-

ric acid TS and shake gently. Then allow the mixture to stand for exactly 2 minutes, add 8 mL of ammonium sulfamate TS and shake occasionally for 5 minutes. Add 3 drops of a solution of 1-naphthol in ethanol (95) (1 in 10), allow to stand for 1 minute, add 3.5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, mix and immediately add water to make 50 mL. Determine within 20 minutes the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared in the same manner, as the blank: the absorbance is not more than 0.25.

(3) ***Soluble halides***—Dissolve 0.5 g of Iotalamic Acid in 20 mL of diluted ammonia TS (1 in 40), add 6 mL of dilute nitric acid, shake, allow to stand for 5 minutes and filter. Transfer the filtrate to a Nessler tube, wash the residue with 20 mL of water, combine the filtrate and the washings and add water to make 50 mL. Proceed as directed for the Chloride Limit Test using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS and add 20 mL of diluted ammonia TS (1 in 40), 6 mL of dilute nitric acid and water to make 50 mL.

(4) ***Iodine***—Dissolve 0.20 g of Iotalamic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mo1/L sulfuric acid TS and allow to stand for 10 minutes with occasional shaking. Add 5 mL of chloroform, shake well and allow to stand: the chloroform layer remains colorless.

(5) ***Heavy metals***—Proceed with 1.0 g of Iotalamic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Arsenic***—Prepare the test solution with 0.6 g of Iotalamic Acid according to Method 3 and perform the test (not more than 3.3 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

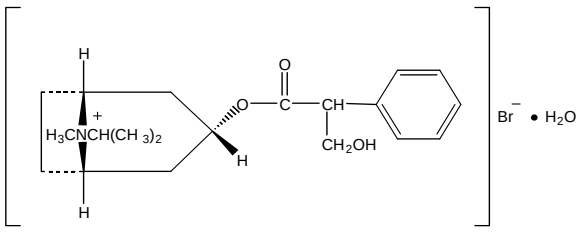
Assay Weigh accurately about 0.4 g of Iotalamic Acid, previously dried, place in a flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder and heat for 30 minutes under a reflux condenser. Cool, filter, wash the flask and the filter paper with 50 mL of water and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution and titrate with 0.1 mol/L silver nitrate VS, until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS
= 20.464 mg of $C_{11}H_9I_3N_2O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ipratropium Bromide Hydrate



$C_{20}H_{30}BrNO_3 \cdot H_2O$: 430.38

(1*R*,3*R*,5*S*,8*R*)-3-[(3-hydroxy-2-phenylpropanoyl)oxy]-8-methyl-8-(propan-2-yl)-8-azabicyclo[3.2.1]octan-8-ium bromide hydrate [*66985-17-9*]

Ipratropium Bromide, when dried, contains not less than 99.0 % and not more than 101.0 % of ipratropium bromide ($C_{20}H_{30}BrNO_3$: 412.36).

Description Ipratropium Bromide is a white, crystalline powder.
Ipratropium Bromide is freely soluble in water, soluble in ethanol (99.5), slightly soluble in acetonitrile or in acetic acid (100) and practically insoluble in ether.

pH—The pH of a solution of Ipratropium Bromide (1 in 20) is between 5.0 and 7.5.

Melting point—About 223 ºC (with decomposition, after drying).

Identification (1) To 5 mg of Ipratropium Bromide, add 0.5 mL of fuming nitric acid and evaporate on a water-bath to dryness. After cooling, dissolve the residue in 5 mL of acetone and add 2 drops of potassium hydroxide-ethanol TS: a purple color develops.

(2) Determine the absorption spectra of solutions of Ipratropium Bromide Hydrate and Ipratropium Bromide Hydrate RS in 0.01 mol/L hydrochloric acid TS (3 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelength. maxima .

(3) Determine the infrared spectra of Ipratropium Bromide Hydrate and Ipratropium Bromide Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumber.

(4) The solution of Ipratropium Bromide Hydrate (1 in 100) responds to the Qualitative Tests for bromide.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ipratropium Bromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 1.0 g of Ipratropium Bromide Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Ipratropium Bromide Hydrate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 2.0 g of Ipratropium Bromide Hydrate according to Method 3 and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 10) (not more than 1 ppm).

(5) ***Isopropylatropine bromide***—Dissolve 25 mg of Ipratropium Bromide Hydrate in the mobile phase to make exactly 100 mL and use this solution as the test solution. Perform the test with 25 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area, A_a, of ipratropium and the peak area, A_b, having a ratio of the retention time to ipratropium about 1.3 by the automatic integration method: $A_b/(A_a + A_b)$ is not more than 0.01 and no peak other than the peak of ipratropium and the peak having a ratio of the retention time to ipratropium about 1.3 appears within about 14 minutes of the retention time after the solvent peak.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 10 cm to 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of diluted phosphoric acid (1 in 200), acetonitrile and methanesulfonic acid (1000 : 120 : 1).

Flow rate: Adjust the flow rate so that the retention time of ipratropium is about 7 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ipratropium obtained from 25 μL of the test solution composes 50 % to 80 % of the full scale.

System performance: Heat a solution of Ipratropium Bromide Hydrate in 1 mol/L hydrochloric acid TS (1 in 100) at 100 ºC for 1 hour and cool. To 2.5 mL of this solution, add the mobile phase to make 100 mL. When the procedure is run with 25 μL of this solution according to the above operating conditions, the resolution, between the peak of ipratropium and the peak having a ratio of the retention time to ipratropium about 0.6, is not less than 3.0.

(6) ***Apo-compounds***—Dissolve 0.14 g of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry and determine the absorbances, A_1 and A_2, at 246 nm and 263 nm, respectively: A_1/A_2 is not more than 0.91.

Loss on Drying 3.9 ~ 4.4 % (1 g, 105 ºC, 4 hours).

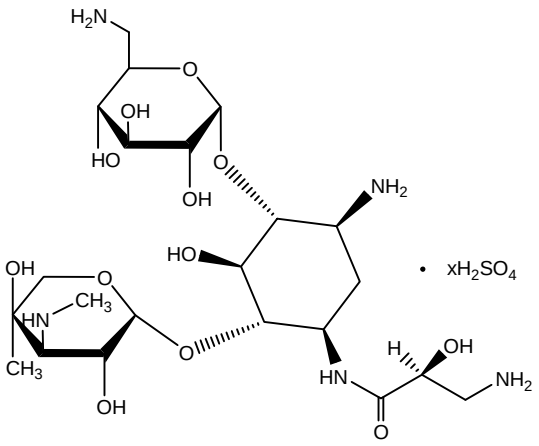
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Ipratropium Bromide Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of 1,4-dioxane and 2.5 mL of bismuth nitrate TS and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Tetrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.24 mg of $C_{20}H_{30}BrNO_3$

Containers and Storage ***Containers***—Tight containers.

Isepamicin Sulfate



$C_{22}H_{43}N_5O_{12}\cdot xH_2SO_4$

[3-Deoxy-4-C-methyl-3-methylamino-b-L-arabinopyranosyl-(1→6)]-2-deoxy-1-*N*-[(2*S*)-3-amino-2-hydroxypropanoyl]-D-streptamine sulfate
[*67814-76-0*]

Isepamicin Sulfate is the sulfate of a derivative of gentamycin B, an aminoglycoside substance, having antibacterial activity produced by the growth of *Micromonospora purpurea*.

Isepamacin Sulfate contains not less than 680 μg (potency) and not more than 780 μg (potency) per mg of isepamicin ($C_{22}H_{43}N_5O_{12}$: 569.60), calculated on the anhydrous basis.

Description Isepamicin Sulfate appears as white to pale yellowish white powder.

Isepamicin Suflate is very soluble in water, and practically insoluble in methanol or in ethanol (95).

Isepamicin Sulfate is hygroscopic.

Identification Dissolve 0.02 g of Isepamicin Sulfate in 1 mL of water, add 3 mL of anthrone TS, shake, and allow to stand: a blue-purple color is produced.

(2) Dissolve 10 mg each of Isepamicin Sulfate and Isepamicin Sulfate RS in 5 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), ethanol (99.5), 1-butanol, and chloroform (5 : 5 : 4 : 2) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS, and heat at about 100 °C for about 10 minutes: the principal spot obtained from the test solution and the spot obtained from the standard solution are red-brown in color and have the same R_f value.

(3) Dissolve 0.01 g of Isepamicin Sulfate in 1 mL of water, and add 1 drop of barium chloride TS: a white precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +100 ~ +120° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.5 g of Isepamicin Sulfate in 5 mL of water is between 5.5 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Isepamicin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Isepamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Perform the test with 5 μL of the test solution used in the Assay as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the test solution by the automatic integration method. Calculate the amount of each related substance by the area percentage method: the amount of HAPA-gentamine B having the relative retention time of about 0.4 with respect to isepamicin is not more than 5.0 %, and the amount of gentamicin B having the relative retention time of about 1.3 with respect to isepamicin is not more than 3.0 %. Correct the peak area of gentamicin B by multiplying the relative response factor, 1.11.

Operating conditions

Detector, column column temperature, reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the test solution, addd water to make exactly 10 mL, and use this solution as the system suitability solution. Pipet 1 mL of this solution, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 μL of this solution is equivalent to 7 to 13 % of that from 5 μL of the system suitability solution.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

Water Not more than 12.0 % (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2 : 1) instead of methanol for water determination.)

Residue on Ignition Not more than 1.0 % (1 g).

Sterility Test It meets the requirement, when Isepamicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of isepamicin, when Isepamicin Sulfate is used in a sterile preparation.

Assay Weigh accurately about 20 mg (potency) each of Isepamicin Sulfate and Isepamicin Sulfate RS, dissolve each in water to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of isepamicin in each solution.

$$\text{Amount [μg (potency)] of isepamicin } (C_{22}H_{43}N_5O_{12}) = \text{Amount [μg (potency)] of Isepamicin Sulfate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Apparatus: Consist of two pumps for the mobile phase and reaction reagent transport, sample injection port, column, reaction coil, detector, and recorder. Use a reaction coil with a thermostat.

Detector: A spectrofluorometer (excitation wavelength: 360 nm, detection wavelength: 440 nm)

Column: A stainless steel column 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Reaction coil: A column 0.25 mm in internal diameter and 5 m in length

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentanesulfonate in about 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reaction reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution

(pH 10.0) add a solution obtained by dissolving 0.4 g of *o*-phthaldehyde in 5 mL of ethanol (95), 1 mL of 2-mercaptoethanol, and 2 mL of a solution of lauromacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45 °C

Flow rate of mobile phase: About 0.6 mL/minute

Flow rate of reaction reagent: About 0.5 mL/minute

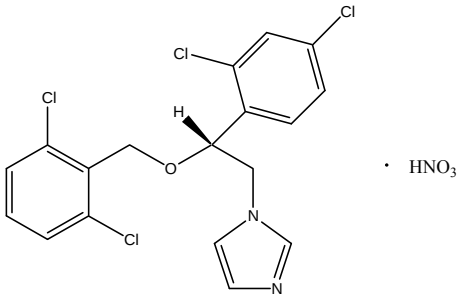
System suitability

System performance: Dissolve 2 mg of gentamicin B in 10 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isepamicin is not more than 3.0 %.

Containers and Storage ***Containers***—Tight containers.

Isoconazole Nitrate



· HNO_3

and enantiomer

$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$: 479.14

1-{2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole; nitric acid [*24168-96-5*]

Isoconazole Nitrate contains not less than 99.0 % and not more than 101.0 % of isoconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$), calculated on the dried basis.

Description Isoconazole Nitrate appears as white powder.

Isoconazole Nitrate is soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Weigh an amount of Isoconazole Nitrate, equivalent to 1 mg of nitric acid, add to a mixture of 0.1 mL of nitrobenzene and 0.2 mL of sulfuric acid and allow to stand for 5 minutes. Cool on ice water, add 5 mL of water slowly with stirring, add 5 mL of 10 mol/L sodium hydroxide TS and 5 mL of acetone, shake, and allowed to stand: the upper layer develops deep purple.

(2) Determine the infrared spectra of Isoconazole Nitrate and Isoconazole Nitrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 30 mg of Isoconazole Nitrate in 5 mL of methanol and use as the test solution. Separately, dissolve 30 mg of Isoconazole Nitrate RS in 5 mL of methanol and use this solution as the standard solution (1). Dissolve 30 mg of Isoconazole Nitrate RS and 30 mg of Econazole Nitrate RS in 5 mL of methanol and use this solution as the standard solution (2). Perform the test with 5 μL each of the test solution and the standard solutions (1) and (2) as directed under Thin Layer Chromatography. Spot 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, 1,4-dioxane and ammonium acetate (40 : 40 : 20) to a distance of about 15 cm and warm air-dry the plate for 15 minutes. Expose this plate to iodine vapor. The principal spots from the test solution and the standard solution show the same color and R_f value. This test is valid only if two spots in the chromatogram obtained with the standard solution (2) separate clearly.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10° (0.2 g, methanol, 20 mL, 100 mm)

Melting Point 178 ~ 182 °C.

Purity (1) ***Clarity and color of solution***—To 0.2 g of Isoconazole Nitrate add methanol to make 20 mL: it is clear.

(2) ***Related substances***—Weigh accurately 0.10 g of Isoconazole Nitrate, dissolve in mobile phase to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 2.5 mg of Isoconazole Nitrate RS and 2.5 mg of Econazole Nitrate RS in mobile phase to make exactly 100 mL and use this solution as the standard solution (1). To 1.0 mL of the test solution add mobile phase to make exactly 100 mL, pipet 5.0 ml of this solution, add mobile phase to make exactly 20 mL and use this solution as the standard solution (2). Perform the test with exactly 10 μL each of the test and standard solutions (2) as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of any peak other than the principal peak in the chromatogram from the test solution is not greater than the area of the principal peak from the standard solution (2) (0.25 %); the total area of all peaks other than the principal peak with the test solution is not greater than twice the area of the principal peak in the chromatogram from the standard

solution (2) (0.5 %). Disregard any peaks due to the solvent. nitrate ion and any peak with an area less than 0.2 times that of the principal peak in the chromatogram from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 10 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (3 μm in particle diameter).

Mobile phase: Dissolve 6 g of ammonium acetate in a mixture of water, methanol and acetonitrile (380 : 320 : 300).

Flow rate: 2.0 mL/minute

System suitability

Detection sensitivity: Adjust so that the height of the principal peak in the chromatogram obtained with 10 μL of the standard solution (2) is at least 50 % of the full scale of the recorder.

System performance: When the procedure is run with 10 μL of the standard solution (1) under the above operating condition, econazole and isoconazole are eluted in this order with the retention times being about 10 minutes and 14 minutes, respectively and resolution between the peaks of econazole and isoconazole is nol less than 5.0.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

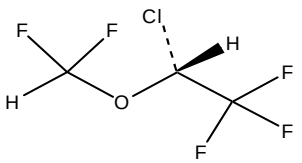
Assay Weigh accurately about 0.35 g of Isoconazole Nitrate, add 75 ml of a mixture of 2-butanone and acetic acid (100) (7 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{15}Cl_4N_3O_4$.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Isoflurane



and enantiomer

$C_3H_2ClF_5O$: 184.49

2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane [*26675-46-7*]

Isoflurane contains not less than 99.0 % and not more than 101.0 % of isoflurane ($C_3H_2ClF_5O$), calculated on the anhydrous basis.

Description Isoflurane is a clear, colorless fluidal liquid.

Isoflurane is slightly soluble in water. Isoflurane is miscible with ethanol (99.5), methanol or o-xylene. Isoflurane is volatile, and has no inflammability.

Isoflurane shows no optical rotation.

Refractive Index— n_D^{20} : about 1.30

Boiling Point—47 ~ 50 ºC.

Identification (1) Determine the infrared spectra of Isoflurane and Isoflurane RS as directed in the liquid film method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The test solution obtained by the Oxygen Flask Combustion Method with 50 μL of Isoflurane, using 40 mL of water as the absorbing liquid, responds to the Qualitative Tests for chloride and fluoride.

Specific Gravity d_{20}^{20} : 1.500 ~ 1.520.

Purity (1) ***Acidity or alkalinity***—To 10 mL of Isoflurane add 5 mL of freshly boiled and cooled water, and shake for 1 minute: the water layer is neutral.

(2) ***Soluble chloride***—To 60 g of Isoflurane add 40 mL of water, shake thoroughly, and separate the water layer. To 20 mL of the water layer add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution directed under Chloride Limit Test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 3 ppm).

(3) ***Soluble fluoride***—To 6 g of Isoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of the 0.01 mol/L sodium hydroxide TS (1 in 20) layer into a Nessler tube, add 30 mL of a mixture of alizarin complexone TS, acetic acid—potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1 : 1 : 1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the test solution. Separately, to 0.4 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a Nessler tube add 30 mL of the mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1 : 1 : 1), then proceed in the same manner as for the preparation of the test solution, and use the solution so obtained as the standard solution. Determine the absorbances of the test solution and the standard solution at 600 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry, using a solution, obtained by proceeding in the same manner as above with

4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), as the blank: the absorbance of the test solution is not more than that of the standard solution (not more than 2 ppm).

Fluorine standard solution—Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(4) ***Related substances***—Use Isoflurane as the test solution. To exactly 1 mL of the test solution add o-xylene to make exactly 100 mL. Pipet 1 mL of this solution, add *o*-xylene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than isoflurane from the test solution is not more than the peak area of isoflurane from the standard solution, and the total area of all peaks other than isoflurane from the test solution is not more than 3 times the peak area of isoflurane from the standard solution.

Operating conditions

Detector, column, column temperature, carrier gas, and flow rate: proceed as directed in the operating conditions in the Assay.

System suitability

System performance and System repeatability : proceed as directed in System suitability under the Assay.

Test for required detectability: To exactly 1 mL of the standard solution add *o*-xylene to make exactly 2 mL. Confirm that the peak area of isoflurane obtained with 5 μL of this solution is equivalent to 35 to 65 % of that with 5 μL of the standard solution.

Time span of measurement: About 5 times as long as the retention time of isoflurane after injection of the test solution.

(5) ***Peroxide***—To 10 mL of Isoflurane add 1 mL of a solution of potassium iodide (1 in 10), freshly prepared, shake vigorously, and allow to stand in a dark place for 1 hour: the water layer is not yellow.

(6) ***Residue on evaporation***—Pipet 65 mL of Isoflurane, evaporate on a water bath, and dry the residue at 105 ºC for 1 hour: not more than 1 mg.

Water Not more than 0.1 % (2 g, coulometric titration).

Assay Pipet 5 mL each of Isoflurane and Isoflurane RS (separately determined water content in the same manner as Isoflurane), add exactly 3 mL of ethyl acetate as the internal standard, then add o-xylene to make exactly 50 mL each. To 5 mL each of these solutions add o-xylene to make 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 2 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of isoflurane to that of the internal standard.

$$\text{Amount (mg) of isoflurane } (C_3H_2ClF_5O) \text{ in 5 mL of Isoflurane} = V_S \times \frac{Q_T}{Q_S} \times 1000 \times 1.506$$

V_S: Amount (mL) of Isoflurane RS, calculated on the anhydrous basis

1.506: Specific gravity (d_{20}^{20}) of isoflurane

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column about 3 mm in internal diameter and about 3.5 m in length, packed with siliceous earth for gas chromatography (between 125 and 149 μm in particle diameter), coated in 10 % with nonylphenoxypoly(ethyleneoxy)-ethanol for gas chromatography and 15 % with polyalkylene glycol for gas chromatography.

Column temperature: A constant temperature of about 80 ºC.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of isolfurane is about 7 minutes.

System suitability

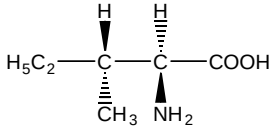
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, isoflurane and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoflurane is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 ºC.

L-Isoleucine



Isoleucine $C_6H_{13}NO_2$: 131.17

(2*S*,3*S*)-2-Amino-3-methylpentanoic acid [*73-32-5*]

L-Isoleucine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-isoleucine ($C_6H_{13}NO_2$).

Description L-Isoleucine appears as white crystals or crystalline powder, is odorless or has a slightly bitter taste.
L-Isoleucine is freely soluble in formic acid, sparingly soluble in water and practically insoluble in ethanol (95) or in ether.
L-Isoleucine dissolves in dilute hydrochloric acid.

Identification Determine the infrared spectra of L-Isoleucine and L-Isoleucine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +39.5 ~ +41.5° (after drying, 1 g, 6 mol/L hydrochloric acid TS. 25 mL, 100 mm).

pH Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the pH of this solution is 5.5 - 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of L-Isoleucine in 20 mL of 1mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool and add water to make 50 mL. Perform the test. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) ***Iron***—Dissolve 0.333 g of L-Isoleucine in water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution, and mix: the color of the test solution is not more intense than that of the standard solution (not more than 30 ppm).

(7) ***Arsenic***—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2 and perform the test. (not more than 2 ppm).

(8) ***Related substances***—Dissolve 0.10 g of L-Isoleucine in 25 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution and add water to make exactly 50 mL. Pipet 5.0 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80 ºC for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 ºC, 3 hours).

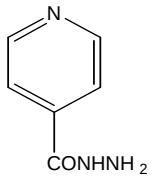
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of L-Isoleucine, previously dried and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-yellow through yellowish green to green (indicator: 0.5 mL of α-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.117 mg of $C_6H_{13}NO_2$

Containers and Storage ***Containers***—Tight containers.

Isoniazid



Isonicotinic Acid Hydrazide $C_6H_7N_3O$: 137.14

Pyridine-4-carbohydrazide [*54-85-3*]

Isoniazid, when dried, contains not less than 98.5 % and not more than 101.0 % of isoniazid ($C_6H_7N_3O$).

Description Isoniazid appears as colorless crystals or a white, crystalline powder and is odorless.
Isoniazid is freely soluble in water, in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride and very slightly soluble in ether.

Identification (1) Dissolve about 20 mg each of Isoniazid and Isoniazid RS in water to make 200 mL and to 5 mL of these solutions, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths..

(2) Determine the infrared spectra of Isoniazid and Isoniazid RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 170 ~ 173 ºC.

pH Dissolve 1.0 g of Isoniazid in 10 mL of freshly boiled and cooled water: the pH of this solution is between 6.5 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Isoniazid in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Isoniazid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 0.40 g of Isoniazid according to Method 3 and perform the test. In this case, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide and ignite the ethanol to burn (not more than 5 ppm).

(4) ***Hydrazine***—Dissolve 0.10 g of Isoniazid in 5 mL of water, add 0.1 mL of a solution of salicylaldehyde in ethanol (95) (1 in 20), shake immediately and allow to stand for 5 minutes: no turbidity is produced.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Isoniazid, previously dried, Dissolve in 50 mL of acetic acid (100) and 10 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 0.5 mL of 1-naphthol-benzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.714 mg of $C_6H_7N_3O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Isoniazid Tablets

Isonicotinic Acid Hydrazide Tablets

Isoniazid Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of Preparation Prepare as directed under Tablets, with Isoniazid.

Identificatlon Take a portion of powdered Isoniazid Tablets, equivalent to 20 mg of Isoniazid according to the labeled amount, add 200 mL of water, shake well and filter. To 5 mL of the filtrate, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 264 nm and 268 nm.

Dissolution Test Perform the test with 1 tablet of Isoniazid Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL or more of the dissolved solution 20 minutes after starting the test and filter through a membrane filter with a pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5.0 mL of the subsequent, add water to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 0.10 g of Isoniazid RS, previously dried at 105 ºC, for 2 hours, dissolve in water to make exactly 100 mL, then pipet 5.0 mL of this solution, add water to make exactly 50 mL and then pipet 5.0 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 267 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Isoniazid Tablets in 20 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of isoniazid ($C_6H_7N_3O$) = $W_S \times \frac{A_T}{A_S} \times \frac{90}{C}$

W_S: Amount (mg) of Isoniazid RS.
C: Labeled amount (mg) of isoniazid ($C_6H_7N_3O$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Isoniazid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.10 g of isoniazid ($C_6H_7N_3O$), add 150 mL of water, shake for 30 minutes, then add water to make exactly 200 mL and filter. Dis-

card the first 10 mL of the filtrate, pipet 5.0 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Isoniazid RS, previously dried at 105 ºC for 2 hours, dissolve in water to make exactly 100 mL. Pipet 5.0 mL of this solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas, A_T and A_S, of Isoniazid of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = \text{Amount (mg) of Isoniazid RS} \times \frac{A_T}{A_S} \times 2$$

Operoting condition

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 6.80 g of monobasic potassium phosphate in water to make 1000 mL. Separately, dissolve 5.76 g of phosphoric acid in water to make 1000 mL. Mix these solutions, to adjust the pH to 2.5. To 400 mL of this solution, add 600 mL of methanol and dissolve 2.86 g of sodium tridodecane sulfonate.

Flow rate: Adjust the flow rate so that the retention time of Isoniazid is about 5 minutes.

System suitability

System performance: Dissolve 5 mg of Isoniazid and 5 mg of isonicotinic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid and isoniazid are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability : When the test is repeated 6 times with 10 μL each of the standard solution under the above operatig conditions, the relative standard deviation of the peak areas of isoniazid is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Isophane Insulin Injection (Aqueous Suspension)

Isophane Insulin Injection (Aqueous Suspension) is an aqueous suspension for injection. Isophane Insulin Injection contains not less than 90.0 % and not more than 110.0 % of the labeled insulin units and not less than 0.01 mg and not more than 0.04 mg of zinc (Zn: 65.41) for each labeled 100 units. When sodium chloride is used in the preparation of Isophane Insulin Injection (Aqueous Suspension), this should be stated on the label.

Method of Preparation Prepare as directed under Injections, with Insulin and Protamine Sulfate. To each 100 mL of Isophane Insulin Injection (Aqueous Suspension), add either 0.38 to 0.63 g of Dibasic Sodium Phosphate Hydrate, 1.4 to 1.8 g of Concentrated Glycerin, 0.15 to 0.17 g of Cresol and 0.06 to 0.07 g of Phenol, or 0.38 to 0.63 g of Dibasic Sodium Phosphate Hydrate, 0.42 to 0.45 g of Sodium Chloride, 0.7 to 0.9g of Concentrated Glycerin and 0.18 to 0.22 g of Cresol.

Description Isophane Insulin Injection (Aqueous Suspension) is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless, clear supernatant liquid and the precipitate returns easily to the suspension state on gentle shaking. When examined microscopically, the precipitate mostly consists of fine, oblong crystals of 5 to 30 μm in major axis and does not contain amorphous substances or large aggregates.

Identification Adjust the pH of Isophan Insulin Injection (Aqueous suspension to Between 2.5 and 3.5 with dilute hydrochloric acid; Particles dissolve and the solution is clear and colorless..

pH 7.0 ~ 7.4

Purity (1) ***Protein***—Perform the test as directed under the Nitrogen Determination: not exceeding 0.85 mg of nitrogen is found for each labeled 100 units.

(2) ***Isophane ratio***— (i) *Buffer solution A*: Dissolve 2.0 g of anhydrous disodium hydrogen phosphate, 16 g of glycerin, 1.6 g of *m*-cresol and 0.65 g of phenol in water to make exactly 200 mL.

(ii) *Buffer solution B*: Dissolve 2.0 g of anhydrous disodium hydrogen phosphate, 4.35 g of sodium chloride, 8.0 g of glycerin and 2.0 g of *m*-cresol in water to make exactly 200 mL.

(iii) *Insulin solution*: Weigh accurately 1000 units of Insulin RS, dissolve in 1.5 mL of diluted hydrochloric acid (1 in 360) and add 5.0 mL of buffer solution A and water to make 20 mL. Adjust the pH to 7.2 with dilute hydrochloric acid or sodium hydroxide TS. The solution is clear. Dilute with water to make exactly 25 mL, The solution is clear and the pH is between 7.1 and 7.4. When it is stated on the label that sodium chloride is used in the preparation, use 5.0 mL of buffer solution B instead of buffer solution A in the above procedure.

(iv) *Protamine solution*: Weigh accurately 50 mg of Protamine Sulfate RS and dissolve in 2 mL of buffer solution A and water to make 8 mL. Adjust the pH to

7.2 with dilute hydrochloric acid or sodium hydroxide TS and dilute with water to exactly 10 mL. The solution is clear and the pH is between 7.1 and 7.4. When it is stated on the label that Sodium Chloride is used in the preparation, use 2 mL of buffer solution B instead of buffer solution A in the above procedure.

(v) *Procedure*: When Isophane Insulin Injection (Aqueous Suspension) contains 40 units per ml, centrifuge a portion of the suspension, measure exactly two 10 mL volumes of the supernatant liquid in two tubes A and B, respectively, add exactly 1 mL of the Insulin solution to tube, A and 1 mL of the protamine solution to tube B, mix the contents of each tube, allow to stand for 10 minutes and determine the turbidity of each mixture by using a photometer or a nephelometer: the turbidity of the mixture in tube B is not greater than that in tube A. When Isophane Insulin Injection (Aqueous Suspension) contains 80 units per ml, measure exactly 5 mL of the supernatant liquid and proceed in the same manner.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 80 EU/100 insulin units.

Determination of Volume of Injection in Containers It meets the requirement.

Assay (1) ***Insulin***—Take Isophane Insulin Injection (Aqueous Suspension), add diluted hydrochloric acid (1 in 100) to adjust pH to about 2.5 and proceed with the clear solution as directed in the Assay under Insulin Injection.

(2) ***Zinc***—Pipet a volume of Isophane Insulin Injection (Aqueous Suspension), equivalent to about 400 units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make exactly 100 mL, dilute, if necessary, with water to contain 0.6 to 1.0 μg of zinc pet mL and use this solution as the test solution. Separately, pipet a volume of standard zinc solution for the Atomic Absorption Spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution according to the Atomic Absorption Spectrophotometry under the following conditions and determine the amount of zinc in the test solution using the analytical curve obtained from the absorbance of the standard solution.

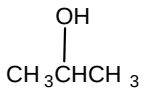
Gas: Dissolved acetylene – Air
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration Date 24 months after preparation.

Isopropanol



Isopropyl Alcohol C_3H_8O: 60.10

Propan-2-ol [*67-63-0*]

Description Isopropanol is a clear, colorless liquid and has a characteristic odor.
Isopropanol is miscible with water, with methanol, with ethanol (95),or with ether.
Isopropanol is flammable and volatile.

Identification (1) To 1 mL of Isopropanol, add 2 mL of iodine TS and 2 mL of sodium hydroxide TS and shake: pale yellow precipitate is formed.

(2) To 5 mL of Isopropanol, add 20 mL of potassium dichromate TS and 5 mL of sulfuric acid with caution and warm gently on a water-bath: the produced gas has the odor of acetone and the gas turns the filter paper, previously wetted with a solution of salicylaldehyde in ethanol (95) (1 in 10) and with a solution of sodium hydroxide (3 in 10), to red brown.

Refractive Index n_D^{20} : 1.376 ~ 1.378.

Specific Gravity d_{20}^{20} : 0.785 ~ 0.788

Purity (1) ***Clarity and of solution***—To 2.0 mL of Isopropanol, add 8 mL of water and shake: the solution is clear.

(2) ***Acid***—To 15.0 mL of Isopropanol, add 50 mL of freshly boiled and cooled water and 2 drops of phenolphthalein TS and add 0.40 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) ***Residue on evaporation***—Evaporate 20.0 mL of Isopropanol on a water-bath to dryness and dry at 105 ºC for 1 hour: the weight of the residue is not more than 1.0 mg.

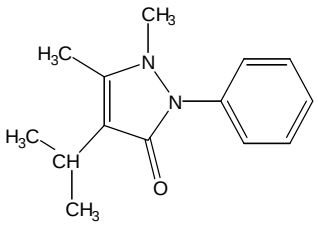
Water Not more than 0.75 % (2 mL, volumetric titration, direct titration).

Distilling Range 81 ~ 83 ºC, not less than 94 vol %.

Containers and Storage ***Containers***—Tight containers.

Storage—Remote from fire.

Isopropylantipyrine



Propyphenazone $C_{14}H_{18}N_2O$: 230.31

1,5-Dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one
[*479-92-5*]

Isopropylantipyrine, when dried, contains not less than 98.0 % and not more than 101.0 % of isopropylantipyrine ($C_{14}H_{18}N_2O$).

Description Isopropylantipyrine appears as white crystals or crystalline powder, is odorless, and has a slightly bitter taste.
Isopropylantipyrine is very soluble in acetic acid (100), freely soluble in ethanol (95) or in acetone, soluble in ether and slightly soluble in water.

Identification (1) To 2 mL of a solution of Isopropylantipyrine (1 in 500), add 1 drop of iron (III) chloride TS: a pale red color develops. Further add 3 drops of sulfuric acid to this solution: the color changes to pale yellow.

(2) Add 5 mL of a solution of Isopropylantipyrine (1 in 500) to a mixture of 5 mL of potassium ferricyanide (III) TS and 1 to 2 drops of iron (III) chloride TS: A dark green color gradually develops.

(3) To 2 mL of a solution of Isopropylantipyrine (1 in 500), add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

Melting Point 103 ~ 105 ºC.

Purity (1) ***Chloride***—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid, 30 mL of dilute ethanol and water to make 50 mL (not more than 0.014 %).

(2) ***Sulfate***—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS, add 1 mL of dilute hydrochloric acid and 30 mL of dilute ethanol and dilute with water to make 50 mL (not more than 0.019 %).

(3) ***Heavy metals***—To 1.0 g of Isopropylantipyrine add 4 mL of a solution of magnesium sulfate heptahydrate in dilute sulfuric acid (1 in 4), mix, and evaporate to dryness by heating in a water bath. Ignite the residue at a temperature not exceeding 800 °C to incinerate. After cooling, moisten the residue with a small amount of dilute sulfuric acid. Evaporate to dryness, and and ignite to incinerate within 2 hours. After cooling, extract the residue with two 5 mL volumes of 2 mol/L hydrochloric acid TS, add 0.1 mL of phenolphthalein TS, and add ammonia solution (28) dropwise until the solution becomes pale red. After cooling, add acetic acid (100) until the color disappears, and add a further 0.5 mL. Filter if necessary, and wash. Add water to make 20 mL, and use this solution as the test solution. Separately, proceed with 1.0 mL of standard lead solution instead of Isopropylantipyrine in the same manner as the test solution. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the control solution. Separately, to 10 mL of water add 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution, add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

System suitability: The control solution shows a slightly brown color compared to the blank solution. To the test solution add 5.0 mL of standard lead solution, and to 10 mL of this solution add 2 mL of the test solution, and use this solution as the system suitability solution. The color of the system suitability solution is not less intense than that of the control solution.

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Isopropylantipyrine according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Antipyrine***—Dissolve 1.0 g of Isopropylantipyrine in 10 mL of dilute ethanol and add 1 mL of sodium nitrite TS and 1 mL of dilute sulfuric acid: no green color develops.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 5 hours).

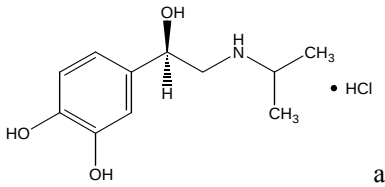
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Isopropylantipyrine, previously dried, dissolve in 60 mL of a mixture of acetic acid (100) and acetic anhydride (2 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry), Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.031 mg of $C_{14}H_{18}N_2O$

Containers and Storage ***Containers***—Tight containers.

Isoproterenol Hydrochloride



and enantiomer

Isoprenaline hydrochloride $C_{11}H_{17}NO_3 \cdot HCl$: 247.72

4-[1-Hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,2-diol hydrochloride [*51-30-9*]

Isoproterenol Hydrochloride, when dried, contains not less than 97.0 % and not more than 101.5 % of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$).

Description Isoproterenol Hydrochloride appears as white, crystalline powder and is odorless.
Isoproterenol Hydrochloride is freely soluble in water, sparingly soluble in ethanol (95) and practically insoluble in acetic acid (100), in acetic anhydride, in chloroform, or in ether.
Isoproterenol Hydrochloride is gradually colored by air and by light.

Identification (1) Determine the absorption spectra of solutions of Isoproterenol Hydrochloride and Isoproterenol Hydrochloride RS in 0.1 mol/L hydrochloric acid TS(1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Isoproterenol Hydrochloride and Isoproterenol Hydrochloride RS, previously dried, as directed in the potassium disk method under Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers.

Melting Point 165 ~ 170 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Isoproterenol Hydrochloride in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.10 g of Isoproterenol Hydrochloride. Prepare the control solution with 0.40 mL of 0.005mol/L sulfuric acid VS (not more than 0.2 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Isoproterenol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Isoproterenone***—Dissolve 50 mg of Isoproterenol Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL and determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry: not more than 0.040.

Loss on Drying Not more than 1.0 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.125 g of Isoproterenol Hydrochloride, dissolve in a solution of sodium bisulfite (3 in 1000), dilute with the solution of sodium bisulfite to make 25 mL and mix. Transfer 5.0 mL of this solution, dilute with 0.17 mol/L acetic acid to make exactly 100 mL and mix. Use this solution as the test solution. Separately, weigh accurately a portion of Isoproterenol Hydrochloride RS to make a solution containing about 2.5 mg per mL and dissolve in mobile phase. Pipet 5.0 mL of this solution and dilute with 0.17 mol/L acetic acid to make exactly 50 mL. Use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Determine, A_T and A_S, the peak areas of isoproterenol for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of isoproterenol hydrochloride } (C_{11}H_{17}NO_3.HCl) = 0.5 \times C \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of Isoproterenol Hydrochloride RS in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: 0.17 mol/L of acetic acid.

Flow rate: 1.5 mL/minute.

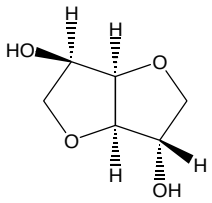
System suitability

System repeatabily: When the test is repeated 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak area of isoproterenol is not more than 3.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Isosorbide



$C_6H_{10}O_4$: 146.14

(3*S*,3a*R*,6*R*,6a*R*)-2,3,3a,5,6,6a-Hexahydrofuro[3,2-b]furan-3,6-diol [*652-67-5*]

Isosorbide, contains not less than 98.5 % and not more than 101.0 % of isosorbide ($C_6H_{10}O_4$), calculated on the anhydrous basis.

Description Isosorbide appears as white crystals or mass, is odorless or has a faint, characteristic odor and has a bitter taste.
Isosorbide is very soluble in water or in methanol, freely soluble in ethanol (95) and slightly soluble in ether.
Isosorbide is hygroscopic.

Identification (1) To 0.1 g of Isosorbide, add 6 mL of diluted sulfuric acid (1 in 2) and dissolve by heating in a water-bath. After cooling, shake well with 1 mL of a solution of potassium permanganate (1 in 30) and heat in a water-bath until the color of potassium permanganate disappears. To this solution, add 10 mL of 2,4-dinitrophenylhydrazine TS and heat in a water-bath: an orange preciptate is formed.

(2) To 2 g of Isosorbide, add 30 mL of pyridine and 4 mL of benzoyl chloride, boil under a reflux condenser for 50 minutes, cool and pour gradually the solution into 100 mL of cold water. Filter the formed precipitate by suction through a glass filter (G3), wash with water, recrystallize twice from ethanol (95) and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts between 102 ºC and 103 ºC.

(3) Determine the infrared spectrum of Isosorbide and Isosorbide TS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: +45.0 ~ +46.0° (5 g, calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Take 25 g of Isosorbide in a Nessier tube and dissolve in 50 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—To a mixture of 1.0 mL of cobalt (II) chloride hexahydrate stock CS, 3.0 mL of iron (III) chloride hexahydrate stock CS and 2.0 mL of cupric sulfate stock CS, add water to make 10.0 mL. To 3.0 mL of this solution, add water to make 50 mL.

(2) ***Sulfate***—Perform the test with 2.0 g of Isosorbide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(3) ***Heavy*** **metals**—Proceed with 5.0 g of Isosorbide according to Method 1 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Isosorbide according to Method 1 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Isosorbide in 10 mL of methanol and use this solution as the test solution. Pipet 2.0 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and cyclohexane (1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a mixture of ethanol (95) and sulfuric acid (9 : 1) on the plate and heat at 105 ºC for 30 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water Not more than 1.5 % (2 g, volumetric titration, direct titration).

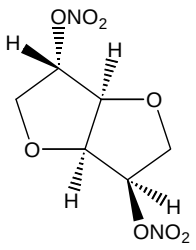
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 10 g of Isosorbide and dissolve in water to make exactly 100 mL. Determine the optical rotation, α_D, of this solution at 20 ± 1 ºC in a 100 mm cell.

Amount (g) of isosorbide ($C_6H_{10}O_4$) = $\alpha_D \times 2.1978$

Containers and Storage ***Containers***—Tight containers.

Isosorbide Dinitrate



$C_6H_8N_2O_8$: 236.14

(3*R*,3a*S*,6*S*,6a*S*)-6-(Nitrooxy)-hexahydrofuro[3,2-b]furan-3-yl nitrate [*87-33-2*]

Isosorbide Dinitrate, contains not less than 95.0 % and not more than 101.0 % of isosorbide dinitrate ($C_6H_8N_2O_8$), calculated on the anhydrous basis.

Description Isosorbide Dinitrate appears as white crystals or crystalline powder, is odorless or has a faint odor like that of nitric acid.
Isosorbide Dinitrate is very soluble in *N,N*-dimethylformamide or in acetone, freely soluble in chloroform or in toluene, soluble in methanol, in ethanol (95) or in ether and practically insoluble in water.
Isosorbide Dinitrate explodes if heated quickly or subjected to percussion.

Identification (1) Dissolve 10 mg of Isosorbide Dinitrate in 1 mL of water and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS and allow to stand for 5 minute to 10 minutes: a brown ring is produced at the zone of contact.

(2) Dissolve 0.1 g of Isosorbide Dinitrate in 6 mL of diluted sulfuric acid (1 in 2) by heating in a water-bath. After cooling, add 1 mL of a solution of potassium permanganate (1 in 30), stir well and heat in a water-bath until the color of potassium permanganate disappears. Add 10 mL of 2,4-dinitrophenylhydrazine TS and heat in a water-bath: an orange precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +134 ~ + 139° (1 g, calculated on the anhydrous basis, ethanol (95), 100 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Isosorbide Dinitrate in 10 mL of acetone: the solution is clear and colorless.

(3) ***Nitrate***—Dissolve 50 mg of Isosorbide Dinitrate in 30 mL of toluene and extract with three 20 mL volumes of water. Combine the aqueous layers and wash with two 20 mL volumes of toluene. To the aqueous layer, add water to make exactly 100 mL and use this solution as the test solution. Pipet 5.0 mL of standard nitric acid solution and 25.0 mL of the test solution in each Nessler tube and add water to make exactly 50 mL, respectively. To each of them, add 60 mg of Griss-Romijin's nitric acid reagent, stir well, allow to stand for 30 minutes and observe from the side of the Nessler tube: the test solution has no more color than the standard solution.

(3) ***Sulfate***—Dissolve 1.5 g of Isosorbide Dinitrate in 15 mL of *N,N*-dimethylformamide, add 60 mL of water, cool and filter. Wash the filter paper with three 20 mL volumes of water, combine the washings with the filtrate and add water to make 150 mL. To 40 mL of this solution, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) ***Heavy metals***—Dissolve 1.0 g of Isosorbide Dinitrate in 30 mL of acetone and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 1.0 mL of standard lead solution, add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

Water Not more than 1.5 % (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Isosorbide Dinitrate in a Kjedahl flask as described under the Nitrogen Deterimination, dissolve in 10 mL of methanol, add 3 g of Devarda's alloy and 50 mL of water and connect the flask with the distillation apparatus as described under the Nitrogen Determination. Measure exactly 25 mL of 0.05 mol/L sulfuric acid VS in an absorption flask, add 5 drops of bromocresol green-methyl red TS and immerse the lower end of the condenser tube in it. Add 15 mL of a solution sodium hydroxide (1 in 2) through the funnel, cautiously rinse the funnel with 20 mL of water, immediately close the clamp attached to the rubber tubing, then begin the distillation until the distillate measures 100 mL. Remove the absorption flask, rinse the end of the condenser tube with a small quantity of water and titrate the distillate and the rinsings with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red through pale red-purple to pale blue-green. Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS
= 11.807 mg of $C_6H_8N_2O_8$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Isosorbide Dinitrate Tablets

Isosorbide Dinitrate Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$: 236.14).

Method of Preparation Prepare as directed under Tablets, with Isosorbide Dinitrate.

Identification Weigh a portion of powdered Isosorbide Dinitrate Tablets equivalent to 0.1 g of lsosorbide Dinitrate, according to the labeled amount, add 50 mL of ether, shake well and filter. Measure 5 mL of the filtrate, evaporate to dryness cautiously, add 1 mL of water to the residue and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS and allow to stand for 5 to 10

minutes: a brown ring is produced at the zone of contact.

Purity ***Nitrate***—Weigh accurately a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 50 mg of Isosorbide Dinitrate according to the labeled amount, transfer to a separator, add 30 mL of toluene, shake thoroughly, extract with three 20 mL volumes of water and proceed as directed in the Purity (2) under Isosorbide Dinitrate.

Disintegration Test It meets the requirement.
For sublingual tablets, the time limit of the test is 2 minutes and omit the use of the auxiliary disk.

Uniformity of Dosage Units It meets the requirement**.**

Assay Weigh accurately and powder not less than 20 Isosorbide Dinitrate. Weigh accurately a portion of the powder, equivalent to about 5 mg of isosorbide dinitrate ($C_6H_8N_2O_8$), dissolve in mobile phase add 5.0 mL of interal standard solution and mobile phase to make exactly 20 mL, filtrate and use this filter as the test solution. Separately, 0.2 g of Isosorbide Nitrate RS (25 %, diluted with lactose), dissolve in mobile phase and to make exactly 50 mL. Pipet 5.0 mL of this solution, add 5.0 mL of internal standard solution and mobile phase to make exactly 20 mL and use this solution as the standard solution. Perform the test with 10μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of Isosorbide Dinitrate to that of the internal standard, for the test solution and the standard solution, respectively.

Amount (mg) of isosorbide dinitrate ($C_6H_8N_2O_8$)
= Amount (mg) of Isosorbide Dinitrate RS

$$\times \frac{C}{100} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

C: Content (%) of isosorbide dinitrate in Isosorbide Dinitrate RS

Internal standard solution—Dissolve 3 g of Nitroglycerin RS (10 % diluted with lactose) in 100 mL of mobile Phase.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm to 30 cm in length, packed with octadeylsilanizd silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: An ordinary temperature.

Mobile phase: To the mixture of methanol and 0.005 mol/L triethylamine solution (50 : 50), add acetic acid to adjust pH to 5.0.

Flow rate: 1.0 mL/minute.

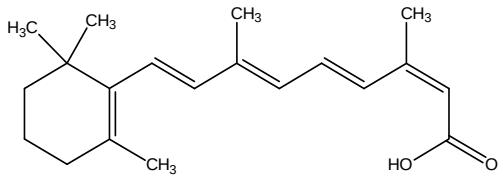
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isosorbide dinitrate and internal standard are eluted in this order with a resolution between their peaks being not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of isosorbide dinitrate to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Isotretinoin



$C_{20}H_{28}O_2$: 300.44

(2*Z*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid [*4759-48-2*]

Isotretinoin contains not less than 98.0 % and not more than 102.0 % of isotretinoin ($C_{20}H_{28}O_2$),calculated on the dried basis.

Description Isotretinoin appears as yellow crystals. Isotretinoin is soluble in chloroform, slightly soluble in ethanol (95), in 2-propanol or in polyethylene glycol 400, and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of a solution of 0.01 mol/L hydrochloric acid TS in 2-propanol (1 in 1000) of Isotretinoin and Isotretinoin RS (1 in 25000) as directed under Ultraviolet-Visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Isotretinoin and Isotretinoin RS as directed under the paste method of Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Isotretinoin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Tretinoin***—Weigh accurately about 0.25 g of

Isotretinoin dissolve, in a small amount of dichloromethane TS, and dilute with isooctane accurately to 100 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of tretinoin RS, dissolve in a small amount of dichloromethane TS, and dilute with isooctane acculately to 100 mL and use this solution as the standard stock solution, add isooctane to 1.0 mL of this solution to make 100 mL and use this solution as the standard solution. Perform the test with each 20 μL of test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine peak areas, A_T and A_S, of tretinoin from the test solution and standard solution by the automatic integration method. The amount of tretinoin is not more than 1.0 %

$$\text{Amount of Tretinoin (\%)} = 10 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of tretinoin in the standard solution.

W: Amount (mg) of Isotretinoin taken.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength : 352 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with porous silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of isooctane, 2-propanol, and acetic acid (100) (99.65 : 0.25 : 0.1)

Flow rate: 1 mL/min

System suitability

System performance**:** Dissolve 25 mg of Isotretinoin RS in a small amount of dichloromethane, dilute with isooctane to make 100 mL and use this solution as the system suitability standard solution. Add the system suitability standard solution to 1 mL of standard stock solution to make 100 mL and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the relative retention times of isotretinoin and tretinoin are 0.84 and 1.0, respectively and the resolution between the peaks of isotretinoin and tretinoin is not less than 2.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak of tretinoin is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, room temperature, vaccum, 16 hours)

Residue on Ignition Not more than 0.1 % (1 g)

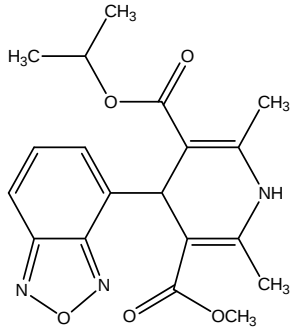
Assay Weigh accurately about 0.24 g of Isotretinoin, dissolve in 50 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L sodium methoxide VS. [indicator: 3 drops of a solution of thymol blue in *N,N*-dimethylformamide (1 in 100)] until the color of solution changes to green. Perform the blank determination and make any necessary correction.

Each ml of 0.1 mol/L sodium methoxide VS
= 30.04 mg of $C_{20}H_{28}O_2$.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and filled with inert gas.

Isradipine



$C_{19}H_{21}N_3O_5$: 371.39

3-Methyl 5-propan-2-yl 4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate
[*75695-93-1*]

Isradipine contains not less than 98.0 % and not more than 102.0 % of isradipine ($C_{19}H_{21}N_5O_5$), calculated on the dried basis.

Description Isradipine appears as yellow, fine crystalline powder.

Isradipine is freely soluble in acetone, soluble in methanol or in acetonitrile, and practically insoluble in n-hexane or in water.

Identification Determine the infrared spectra of Isradipine and Isradipine RS as directed in the potassium disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 166 ~ 170 ºC.

Purity (1) ***Heavy metals*—**Proceed with 1.0 g of Isradipine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances*—**Weigh exactly 50 mg of Isradipine, dissolve in 5.0 mL of methanol, add the mobile phase to make exactly 25 mL, and use this solution as the test solution. Weigh exactly 6 mg of Isradipine RS, dissolve in 5 mL of methanol, add the

mobile phase to make exactly 100 mL, add the mobile phase to 5.0 mL of this solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with each 25 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak area other than isradipine peak from the test solution: the total area of each peak other than Isradipine peak obtained from the test solution is not greater than 4 times the peak area of isradipine obtained from the standard solution (1.2 %), the area of the largest peak other than isradipine peak obtained from the test solution is not greater than 1.6 times the peak area of isradipine obtained from the standard solution (0.5 %), the area of each peak other than the peak area of isradipine obtained from the test solution is not greater than the peak area of isradipine obtained from the standard solution (0.3 %).

Operating conditions

Detector: An ultraviolet-visible spectrophotometry (wavelength: 230 nm).

Mobile phase: A mixture of water, ethanol and tetrahydrofuran (50 : 40 : 10).

Column: A stainless steel column, about 4.6 mm in internal diameter and 10 cm in length, packed with porous silica gel for liquid chromatography (3 μm to 10 μm in particle diameter)

Flow rate: 1.7 mL/minute

System suitability

System performance: Weigh 0.2 g of Isradipine RS and 10 mg of Isradipine related substance I RS, dissolve in 5 mL of methanol, add the mobile phase to make 100 mL. Add the mobile phase to 5.0 mL of this solution to make 50 mL, and use this solution as the solution of the system suitability. When the procedure is run test with 25 μL of this solution as directed under Liquid Chromatography according to the following operating conditions, the resolution between the peak of isradipine and the peak of isradipine related substance I is not less than 1.5 %.

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution of the system suitability under the above operating conditions, the relative standard deviation of the peak area of isradipine is not more than 1.5 %.

Time span of measurement: Not less than 3 times as long as the retention time of Isradipine peak.

Loss on Drying Not more than 0.2 % (105 ºC, 4 hours, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Use the light-resistant containers in this procedure. Weigh accurately about 20 mg of Isradipine, dissolve in 20 mL of methanol, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately a constant mass of Isradipine RS and Isradipine related substances I [isopropylmethyl 4-(4-benzofurzanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate] RS, dissolve in methanol, add the mobile phase to make the solutions containing 0.2 mg/mL and 10 μg /mL respectively, and use these solutions as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions: determine the peak areas, A_T and A_S, of Isradipine .

$$\text{Amount (mg) of isradipine } C_{19}H_{21}N_3O_5) = 100 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Isradipine in the standard solution.

Operating conditions

Detector: An ultraviolet-visible absorption photometer (wavelength : 326 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A misture of water , methanol and tetrahydrofuran (50:40:10)

Flow rate: 1.7 mL/minute

Flow rate: 1.7 mL/minute

System suitability

System performance: When the procedure is run with 25 μL of the system suitability under the above operating conditions, the resolution between isradipine peak and isradipine related substance peak are not more than 1.5.

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isradipine is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.