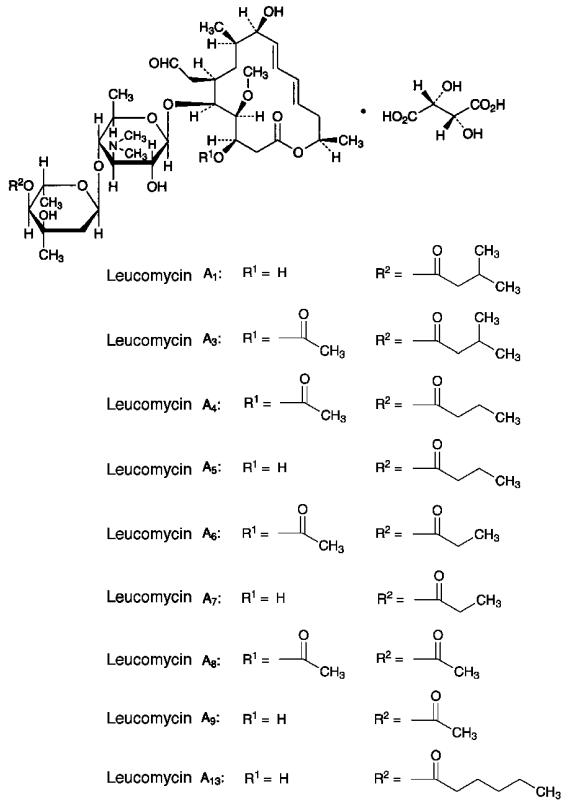
Kitasamycin Tartrate

Leucomycin Tartrate

キタサマイシン酒石酸塩



(Leucomycin A_1, A_5, A_7, A_9 and A_{13} Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A_1 Tartrate: acyl = 3-methylbutanoyl
Leucomycin A_5 Tartrate: acyl = butanoyl
Leucomycin A_7 Tartrate: acyl = propanoyl
Leucomycin A_9 Tartrate: acyl = acetyl
Leucomycin A_{13} Tartrate: acyl = hexanoyl

(Leucomycin A_3, A_4, A_6 and A_8 Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A_3 Tartrate: acyl = 3-methylbutanoyl
Leucomycin A_4 Tartrate: acyl = butanoyl
Leucomycin A_6 Tartrate: acyl = propanoyl
Leucomycin A_8 Tartrate: acyl = acetyl

[*37280-56-1*, Kitasamycin Tartrate]

Kitasamycin Tartrate is the tartrate of kitasamycin. It contains not less than 1300 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin Tartrate occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of *n*-butyl acetate, shake well, and discard the *n*-butyl acetate layer. To the aqueous layer add 20 mL of *n*-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Tests <*1.09*> (1) for tartrate.

pH <*2.54*> Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

Content ratio of the active principle Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amount of leucomycin A_5 is 40 – 70%, leucomycin A_4 is 5 – 25%, and leucomycin A_1 is 3 – 12%. The relative retention times of leucomycin A_4 and leucomycin A_1 with respect to leucomycin A_5 are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 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: To a suitable amount of a solution of ammonium acetate (77 in 5000) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 20 mg each of Leucomycin A_5 RS and Josamycin RS in 20 mL of diluted aceto-

nitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin 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 of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A_5 is not more than 1.0%.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Kitasamycin Tartrate 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).

Water ⟨*2.48*⟩ Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

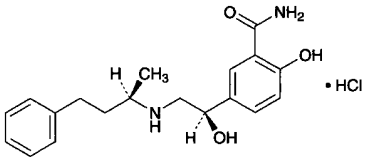
(iii) Standard solutions—Weigh accurately an amount of Leucomycin A_5 RS, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

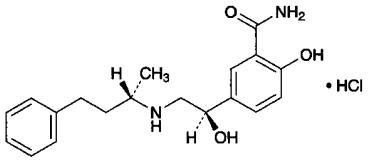
Containers and storage Containers—Tight containers.

Labetalol Hydrochloride

ラベタロール塩酸塩



and enantiomer



and enantiomer

$C_{19}H_{24}N_2O_3.HCl$: 364.87

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*RS*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*SR*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

[*32780-64-6*]

Labetalol Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{19}H_{24}N_2O_3.HCl$.

Description Labetalol Hydrochloride occurs as a white crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

It dissolves in 0.05 mol/L sulfuric acid TS.

Melting point: about 181°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Labetalol Hydrochloride in 0.05 mol/L sulfuric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Labetalol Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Labetalol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.5 g of Labetalol Hydrochloride in 50 mL of water is between 4.0 and 5.0.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Labetalol 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 0.8 g of Labetalol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨2.03⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution do not exceed 2 in number and are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨2.41⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Labetalol Hydrochloride in 0.7 mL of a solution of *n*-butylboronic acid in anhydrous pyridine (3 in 250), allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography ⟨2.02⟩ according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, where A_a is the peak area of the shorter retention time and A_b is the peak area of the longer retention time, using the automatic integration method: the ratio $A_b/(A_a + A_b)$ is between 0.45 and 0.55.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 25 m in length, coated inside with methyl silicone polymer for gas chromatography in 5 μm thickness.

Column temperature: A constant temperature of about 290°C.

Injection port temperature: A constant temperature of about 350°C.

Detector temperature: A constant temperature of about 350°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of labetalol is about 9 minutes.

System suitability—

System performance: Proceed with 2 μL of the sample solution under the above conditions: the resolution between the two labetalol peaks is not less than 1.5.

System repeatability: Repeat the test 6 times under the above conditions with 2 μL of the sample solution: the relative standard deviation of the ratio of the peak area of labetalol with the shorter retention time to that of the longer retention time is not more than 2.0%.

Assay Weigh accurately about 0.3 g of Labetalol Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.49 mg of $C_{19}H_{24}N_2O_3$.HCl

Containers and storage Containers—Tight containers.

Labetalol Hydrochloride Tablets

ラベタロール塩酸塩錠

Labetalol Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl: 364.87).

Method of preparation Prepare as directed under Tablets, with Labetalol Hydrochloride.

Identification (1) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 5 mg of Labetalol Hydrochloride according to the labeled amount, add 100 mL of 0.05 mol/L sulfuric acid TS, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: it exhibits a maximum between 300 nm and 304 nm.

(2) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 0.25 g of Labetalol Hydrochloride according to the labeled amount, add 25 mL of methanol, shake vigorously for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of labetalol hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test using these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 5 μL each of the sample 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, 2-propanol, water, and ammonia solution (28) (25:15:8:2) 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 sample solution and the spot obtained from the standard solution show the same *R*f value.

Uniformity of dosage units ⟨6.02⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Labetalol Hydrochloride Tablets add 5 mL of 0.5 mol/L sulfuric acid TS and 30 mL of water, shake vigorously for 30 minutes, add water to make exactly 50 mL, and filter. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.05 mol/L sulfuric acid TS to make exactly *V* mL so that each mL contains about 40 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 302 nm as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩.

Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl)
$= M_S \times A_T/A_S \times V/40$

M_S: Amount (mg) of labetalol hydrochloride for assay

Dissolution ⟨6.10⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900

mL of water as the dissolution medium, the dissolution rate in 30 minutes of Labetalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Labetalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at specified minute 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, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 50 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, 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 the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 302 nm.

Dissolution rate (%) with respect to the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of labetalol hydrochloride for assay
C: Labeled amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Labetalol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl), add 100 mL of 0.5 mol/L sulfuric acid TS and 600 mL of water, shake vigorously for 30 minutes, add water to make exactly 1000 mL, and filter. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add 0.05 mol/L sulfuric acid TS to make exactly 25 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 302 nm.

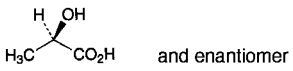
Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl)
$= M_S \times A_T/A_S \times 25$

M_S: Amount (mg) of labetalol hydrochloride for assay

Containers and storage Containers—Tight containers.

Lactic Acid

乳酸



and enantiomer

$C_3H_6O_3$: 90.08
(2*RS*)-2-Hydroxypropanoic acid
[*50-21-5*]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of $C_3H_6O_3$.

Description Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.

Specific gravity d_{20}^{20}: about 1.20

Identification A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests <*1.09*> for lactate.

Purity **(1)** Chloride <*1.03*>—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <*1.14*>—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <*1.07*>—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. 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 from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add

dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, add 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluensulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make exactly 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

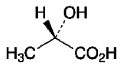
Assay Weigh accurately about 3 g of Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 90.08 mg of $C_3H_6O_3$

Containers and storage Containers—Tight containers.

L-Lactic Acid

L-乳酸



$C_3H_6O_3$: 90.08
(2*S*)-2-Hydroxypropanoic acid
[*79-33-4*]

L-Lactic Acid is a mixture of L-lactic acid and L-lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of $C_3H_6O_3$.

Description L-Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, no unpleasant odor.

It is miscible with water, with ethanol (99.5) and with diethyl ether.

It is hygroscopic.

Specific gravity d_{20}^{20}: about 1.20

Identification A solution of L-Lactic Acid (1 in 50) changes the color of blue litmus paper to red, and responds to the Qualitative Tests ⟨*1.09*⟩ for lactate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −46 – −52° Weigh accurately an amount of L-Lactic Acid, equivalent to about 2 g of L-lactic acid ($C_3H_6O_3$), add exactly 25 mL of 1 mol/L sodium hydroxide VS, cover with a watch glass, and heat on a water bath for 15 minutes. Cool, and adjust to pH 7.0 with 1 mol/L hydrochloric acid VS. Dissolve 5.0 g of hexaammonium heptamolybdate tetrahydrate in this solution, add water to make exactly 50 mL, and determine the optical rotation using a 100-mm cell.

Purity **(1)** Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of L-Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate ⟨*1.14*⟩—Perform the test with 2.0 g of L-Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metal ⟨*1.07*⟩—To 2.0 g of L-Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. 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 from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron ⟨*1.10*⟩—Prepare the test solution with 4.0 g of L-Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of L-Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of L-Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannitol—Shake 10 mL of L-Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm L-Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of L-Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color develops, then add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, and 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of L-Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 3 g of L-Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1

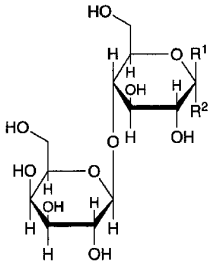
mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 90.08 mg of $C_3H_6O_3$

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖



α-Lactose : R^1=H, R^2=OH
β-Lactose : R^1=OH, R^2=H

$C_{12}H_{22}O_{11}$: 342.30
β-D-Galactopyranosyl-(1→4)-β-D-glucopyranose (β-lactose)
β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose (α-lactose)
[*63-42-3*, Anhydrous Lactose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Anhydrous Lactose is β-lactose or a mixture of β-lactose and α-lactose.

◆The relative quantities of α-lactose and β-lactose in Anhydrous Lactose is labeled as the isomer ratio.◆

◆**Description** Anhydrous Lactose occurs as white crystals or powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

◆**Identification** Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Anhydrous Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +54.4 – +55.9°. Weigh accurately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

◆**(3)** Heavy metals <*1.07*>—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).◆

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 80°C, 2 hours).

Water <*2.48*> Not more than 1.0% (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

◆**Microbial limit** <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 5×10^1 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

Isomer ratio Place 1 mg of Anhydrous Lactose in a 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (18:7), seal the vial tightly with a screw cap, and mix gently. Allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the peak areas of α-lactose and β-lactose, A_a and A_b, and calculate the contents (%) of α-lactose and β-lactose in Anhydrous Lactose by the following equations.

Content (%) of α-lactose = $A_a/(A_a + A_b) \times 100$
Content (%) of β-lactose = $A_b/(A_a + A_b) \times 100$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Injection port temperature: A constant temperature of about 275°C.

Detector temperature: A constant temperature of about 275°C.

Column: A glass column 4 mm in inside diameter and 90 cm in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.

Carrier gas: Helium.

Flow rate: A constant flow rate of about 40 mL per minute.

System suitability—

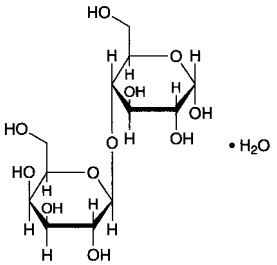
System performance: Prepare a solution with 1 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose: the relative retention time of α-lactose with respect to that of β-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

◆**Containers and storage** Containers—Well-closed containers.◆

Lactose Hydrate

Lactose

乳糖水和物



$C_{12}H_{22}O_{11}.H_2O$: 360.31

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate

[*64044-51-5*, Mixture of α- and β-lactose monohydrate]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Lactose Hydrate is the monohydrate of β-D-galactopyranosyl-(1→4)-α-D-glucopyranose.

◆It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.◆

◆The label states the effect where it is the granulated powder.◆

◆**Description** Lactose Hydrate occurs as white, crystals, powder or granulated powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Lactose Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with ◆the Reference Spectrum or◆ the spectrum of Lactose Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +54.4 – +55.9°. Weigh accurately about 10 g of Lactose Hydrate, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

◆**(3)** Heavy metals <*1.07*>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).◆

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

◆**Loss on drying** <*2.41*> Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).◆

Water <*2.48*> 4.5 – 5.5%. ◆For the granulated powder, 4.0 – 5.5%◆ (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

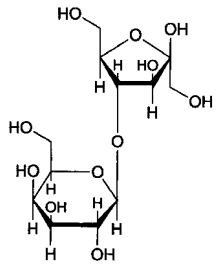
Residue on ignition <*2.44*> Not more than 0.1% (1 g).

◆**Microbial limit** <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 5×10^1 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

◆**Containers and storage** Containers—Well-closed containers.◆

Lactulose

ラクツロース



$C_{12}H_{22}O_{11}$: 342.30
β-D-Galactopyranosyl-(1→4)-D-fructose
[*4618-18-2*]

Lactulose is a solution of lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.

It contains not less than 50.0% and not more than 56.0% of $C_{12}H_{22}O_{11}$.

Description Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste.

It is miscible with water and with formamide.

Identification **(1)** To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 0.5 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling's TS, and boil for 5 minutes: a red precipitate is produced.

pH <*2.54*> To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

Specific gravity <*2.56*> d_{20}^{20}: 1.320 – 1.360

Purity **(1)** Heavy metals <*1.07*>—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

(3) Galactose and lactose—Determine the heights of the peaks corresponding to galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of galactose and lactose to that of the internal standard from the sample solution, Q_{Ta} and Q_{Tb}, and then from the standard solution, Q_{Sa} and Q_{Sb}: it contains galactose of not more than 11%, and lactose of not more than 6%.

$$\text{Amount (mg) of galactose } (C_6H_{12}O_6) = M_S \times Q_{Ta}/Q_{Sa}$$

M_S: Amount (mg) of galactose

$$\text{Amount (mg) of lactose } (C_{12}H_{22}O_{11}.H_2O) = M_S \times Q_{Tb}/Q_{Sb}$$

M_S: Amount (mg) of lactose Hydrate

Loss on drying <*2.41*> Not more than 35% (0.5 g, in vacuum, 80°C, 5 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose RS, accurately about 80 mg of D-galactose and accurately about 40 mg of lactose monohydrate, add exactly 10 mL of the 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of lactulose to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{12}H_{22}O_{11} = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Lactulose RS

Internal standard solution—A solution of D-mannitol (1 in 20).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 50 cm in length, packed with gel type strong acid ion-exchange resin for liquid chromatography (degree of cross-linkage: 6%) (11 μm in particle diameter).

Column temperature: A constant temperature of about 75°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of lactulose is about 18 minutes.

System suitability—

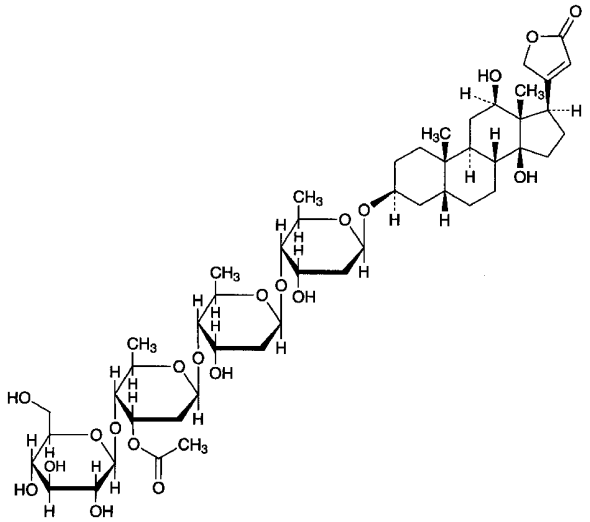
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lactulose and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak heights of lactulose, galactose and lactose to the height of the internal standard are not more than 2.0%, respectively.

Containers and storage Containers—Tight containers.

Lanatoside C

ラナトシド C



$C_{49}H_{76}O_{20}$: 985.12

3β-[β-D-Glucopyranosyl-(1→4)-3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide

[*17575-22-3*]

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of $C_{49}H_{76}O_{20}$.

Description Lanatoside C occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring is produced, and the color of the upper layer near the contact zone gradually changes to blue through purple. Finally the color of the entire acetic acid layer changes to blue-green through deep blue.

Purity Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <*2.03*> with these solutions. Spot 20 μL each of the sample solution and 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: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +32 – +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

Loss on drying <*2.41*> Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C RS, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, A_T and A_S, of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

$$\text{Amount (mg) of } C_{49}H_{76}O_{20} = M_S \times A_T/A_S$$

M_S: Amount (mg) of Lanatoside C RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Lanatoside C Tablets

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Lanatoside C Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$: 985.12).

Method of preparation Prepare as directed under Tablets, with Lanatoside C.

Identification **(1)** Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C according to the labeled amount, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

(2) Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography <*2.03*>. Spot 25 μL each of these solutions 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 obtained from the sample solution and standard solution show a black color, and have the same *R*f values.

Uniformity of dosage unit ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic waves, add ethanol (95) to make exactly V mL of a solution containing about 5 μg of lanatoside C ($C_{49}H_{76}O_{20}$) in each mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 2 mL of this solution, add 10 mL of water, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 40 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of the subsequent solutions from the sample solution and the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry ⟨*2.22*⟩, respectively.

$$\text{Amount (mg) of lanatoside C } (C_{49}H_{76}O_{20}) = M_S \times (F_T - F_B)/(F_S - F_B) \times V/5000$$

M_S: Amount (mg) of Lanatoside C RS

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rate in 60 minutes of Lanatoside C Tablets is not less than 65%. No retest requirement is applied to Lanatoside C Tablets.

Start the test with 1 tablet of Lanatoside C Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 0.5 μg of lanatoside ($C_{49}H_{76}O_{20}$) according to the labeled amount, and use this solution as the sample solution. Separately, dry Lanatoside C RS in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of diluted hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, F_T, F_S and F_B, of the sample solution and the standard solution at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry ⟨*2.22*⟩.

$$\text{Dissolution rate (\%) with respect to the labeled amount of lanatoside C } (C_{49}H_{76}O_{20}) = M_S \times (F_T - F_B)/(F_S - F_B) \times V'/V \times 1/C$$

M_S: Amount (mg) of Lanatoside C RS

C: Labeled amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Lanatoside C Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of lanatoside C ($C_{45}H_{76}O_{20}$), into a 100-mL light-resistant volumetric flask, add 50 mL of ethanol (95), and shake for 15 minutes. Then dilute with ethanol (95) to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 5 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, dissolve in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution into light-resistant, glass-stoppered test tubes, add exactly 3 mL each of alkaline 2,4,6-trinitrophenol TS, shake well and allow these solutions to stand between 22°C and 28°C for 25 minutes. Determine the absorbances, A_T and A_S, of the subsequent sample solution and the subsequent standard solution at 490 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared by the same manner with 5 mL of ethanol (95), as the blank.

$$\text{Amount (mg) of lanatoside C } (C_{49}H_{76}O_{20}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Lanatoside C RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Hydrous Lanolin

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Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C.

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact,

and sulfuric acid layer shows a green fluorescence.

Acid value ⟨*1.13*⟩ Not more than 1.0.

Iodine value 18 – 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\text{Iodine value} = (a - b) \times 1.269/M$$

M: amount (g) of sample
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration

Purity **(1)** Acidity or alkalinity—To 5 g of Hydrous Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride ⟨*1.03*⟩—To 2.0 g of Hydrous Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate 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 with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of the dried residue obtained in the Residue on evaporation in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. Add dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observed in the same level with the spot of standard solution. For this test use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Residue on evaporation Weigh accurately about 12.5 g of Hydrous Lanolin, dissolve in 50 mL of diethyl ether, place it in a separator, transfer the separated aqueous layer to another separator, add 10 mL of diethyl ether, shake, and combine the diethyl ether layer and diethyl ether in the first separator. Shake the diethyl ether layer with 3 g of anhydrous sodium sulfate, and filter through dry filter paper. Wash the separator and the filter paper with two 20-mL portions of diethyl ether, combine the washings with the filtrate, evaporate on a water bath until the odor of diethyl ether is no longer perceptible, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the content is not less than 70% and not more than 75%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Purified Lanolin

Adeps Lanae Purificatus

精製ラノリン

Purified Lanolin is the purified product of the fat-like substance obtained from the wool of *Ovis aries* Linné (*Bovidae*).

Description Purified Lanolin is a light yellow to yellowish brown, viscous, ointment-like substance, and has a faint, characteristic but not rancid odor.

It is very soluble in diethyl ether and in cyclohexane, freely soluble in tetrahydrofuran and in toluene, and very slightly soluble in ethanol (95). It is practically insoluble in water, but miscible without separation with about twice its mass of water, retaining ointment-like viscosity.

Melting point: 37 – 43°C

Identification Superimpose carefully 1 mL of a solution of Purified Lanolin in cyclohexane (1 in 50) on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and the sulfuric acid layer shows a green fluorescence.

Acid value ⟨*1.13*⟩ Not more than 1.0.

Iodine value 18 – 36 Weigh accurately about 0.8 g of Purified Lanolin in a glass-stoppered 500-mL flask, add 20 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus' TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in light-resistant, well-closed containers, with occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = (a - b) \times 1.269/M$$

M: amount (g) of sample.
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the blank determination.
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the titration of the sample.

Purity **(1)** Acid or alkali—To 5 g of Purified Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer:

the aqueous layer is neutral.

(2) Chloride <*1.03*>—To 2.0 g of Purified Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate 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 with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of Purified Lanolin in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observable same level of the spot of standard solution. Use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Total ash <*5.01*> Not more than 0.1%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Lard

Adeps Suillus

豚脂

Lard is the fat obtained from *Sus scrofa* Linné var. *domesticus* Gray (*Suidae*).

Description Lard occurs as a white, soft, unctuous mass, and has a faint, characteristic odor and a bland taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: 36 – 42°C

Congealing point of the fatty acids: 36 – 42°C

Acid value <*1.13*> Not more than 2.0.

Saponification value <*1.13*> 195 – 203

Iodine value <*1.13*> 46 – 70

Purity **(1)** Moisture and coloration—Melt 5 g of Lard by heating on a water bath: it forms a clear liquid, from which no water separates. Observe the liquid in a layer 10 mm thick: the liquid is colorless to slightly yellow.

(2) Alkalinity—To 2.0 g of Lard add 10 mL of water, melt by warming on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: the layer is colorless.

(3) Chloride <*1.03*>—To 1.5 g of Lard add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the opalescence of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, and add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

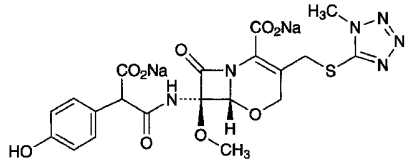
(4) Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal plates grouped irregularly, and do not contain prisms or needles grouped in fan-shaped clusters.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Latamoxef Sodium

ラタモキセフナトリウム



$C_{20}H_{18}N_6Na_2O_9S$: 564.44

Disodium (6*R*,7*R*)-7-[2-carboxylato-2-(4-hydroxyphenyl)acetylamino]-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*64953-12-4*]

Latamoxef Sodium contains not less than 830 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef ($C_{20}H_{20}N_6O_9S$: 520.47).

Description Latamoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.5 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -32 – $-40°$ (0.5 g calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 50 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS and 36 mL of Iron (III) Chloride CS add 11 mL of diluted dilute hydrochloric acid (1 in 10). To 2.5 mL of this solution add 7.5 mL of diluted dilute hydrochloric acid (1:10).

(2) Heavy metals ⟨*1.07*⟩—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed 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 ⟨*1.11*⟩—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1*H*-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not larger than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not larger than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1*H*-tetrazole-5-thiol after multiplying by its ralative response factor, 0.52.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

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

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 latamoxef is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (0.5 g, volumetric titration, back titration).

Isomer ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas, A_a and A_b, of the two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: A_a/A_b is between 0.8 and 1.4.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 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 7.7 g of ammonium acetate in 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 the first eluted peak of latamoxef is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium RS, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of latamoxef to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of latamoxef } (C_{20}H_{20}N_6O_9S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Latamoxef Ammonium RS

Internal standard solution—A solution of *m*-cresol (3 in 200).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 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 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 exactly 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 latamoxef 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, latamoxef 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 5°C.

Lauromacrogol

Polyoxyethylene Lauryl Alcohol Ether

ラウロマクロゴール

Lauromacrogol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with laury alcohol.

Description Lauromacrogol is a colorless or light yellow, clear liquid or a white, petrolatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritative taste.

It is very soluble in ethanol (95), in diethyl ether and in carbon tetrachloride.

It is freely soluble or dispersed as fine oily drops in water.

Identification **(1)** Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.

(2) Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the Solution method under Infrared Spectrophotometry <*2.25*> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 cm^{-1}, 1246 cm^{-1} and 1110 cm^{-1}.

Purity **(1)** Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

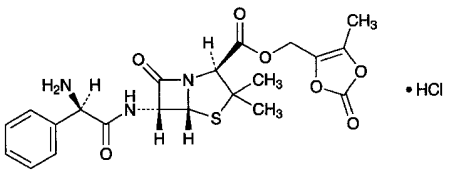
(2) Unsaturated compound—Shake 0.5 g of Lauromacrogol with 10 mL of water, and add 5 drops of bromine TS: the color of the solution does not disappear.

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Containers and storage Containers—Tight containers.

Lenampicillin Hydrochloride

レナンピシリン塩酸塩



$C_{21}H_{23}N_3O_7S.HCl$: 497.95

5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride

[*80734-02-7*]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methyloxodioxolenylmethyl ester.

It contains not less than 653 μg (potency) and not more than 709 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (95), and freely soluble in *N*,*N*-dimethylformamide.

Identification **(1)** Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +174 – +194° (0.2 g calculated on the anhydrous basis and corrected on the amount of residual solvent, ethanol (95), 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Lenampicillin 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 <*1.11*>—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloridein, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the

sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of ampicillin to that of the internal standard: the amount of ampicillin is not more than 1.0%.

$$\text{Amount (\%) of ampicillin } (C_{16}H_{19}N_3O_4S) = M_S/M_T \times Q_T/Q_S \times 2$$

M_S: Amount [mg (potency)] of Ampicillin RS
M_T: Amount (mg) of the sample

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4 mm in inside 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: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ampicillin 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, ampicillin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid ($C_{16}H_{21}N_3O_5S$: 367.42) is not more than 3.0%.

$$\text{Each mL of 0.01 mol/L sodium thiosulfate VS} = 0.45 \text{ mg of } C_{16}H_{21}N_3O_5S$$

(5) Residual solvent <*2.46*>—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 μL each of the sample solution, standard solution (1) and (2) as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, Q_{Sa1} and Q_{Sb1}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, Q_{Sa2} and Q_{Sb2}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

$$\text{Amount (\%) of 2-propanol} = M_{Sa}/M_T \times (2Q_{Ta} - 3Q_{Sa1} + Q_{Sa2})/(Q_{Sa2} - Q_{Sa1})$$

$$\text{Amount (\%) of ethyl acetate} = M_{Sb}/M_T \times (2Q_{Tb} - 3Q_{Sb1} + Q_{Sb2})/(Q_{Sb2} - Q_{Sb1})$$

M_{Sa}: Amount (g) of 2-propanol
M_{Sb}: Amount (g) of ethyl acetate
M_T: Amount (g) of the sample

Internal standard solution—A solution of cyclohexane in *N*,*N*-dimethylformamide (1 in 1000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with tetrakishydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.
Column temperature: A constant temperature of about 80°C.
Injection port temperature: A constant temperature of about 160°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.
System suitability—
System performance: When the procedure is run with 4 μL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.
System repeatability: When the test is repeated 3 times with 4 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

Water <*2.48*> Not more than 1.5% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride RS, equivalent to about 0.1 g (potency), dissolve each in the internal stand-

ard solution to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lenampicillin to that of the internal standard.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) = $M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Lenampicillin Hydrochloride RS

Internal standard solution—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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: Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.

System suitability—

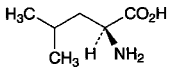
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

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

Containers and storage Containers—Tight containers.

L-Leucine

L-ロイシン



$C_6H_{13}NO_2$: 131.17
(2*S*)-2-Amino-4-methylpentanoic acid
[*61-90-5*]

L-Leucine, when dried, contains not less than 98.5% of $C_6H_{13}NO_2$.

Description L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Leucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +14.5 – +16.0° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Leucine 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 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 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 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 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 with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Leucine 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).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Leucine in water by warming, after cooling, add water to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 <2.03>. Spot 5 μL each of the sample solution and 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 evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.13 g of L-Leucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

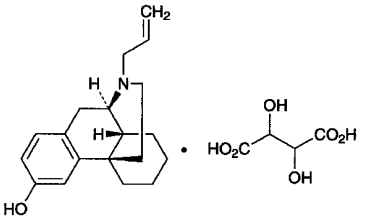
Each mL of 0.1 mol/L perchloric acid VS = 13.12 mg of $C_6H_{13}NO_2$

Containers and storage Containers—Well-closed contain-

ers.

Levallorphan Tartrate

レバロルファン酒石酸塩



$C_{19}H_{25}NO.C_4H_6O_6$: 433.49
17-Allylmorphinan-3-ol monotartrate
[*71-82-9*]

Levallorphan Tartrate, when dried, contains not less than 98.5% of $C_{19}H_{25}NO.C_4H_6O_6$.

Description Levallorphan Tartrate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levallorphan Tartrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <*1.09*> (1) and (2) for tartrate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -37.0 – $-39.2°$ (after drying, 0.2 g, water, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

Melting point <*2.60*> 174 – 178°C

Purity **(1)** Clarity and color of solution—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Levallorphan Tartrate 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 Levallorphan Tartrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia TS (200:3) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.35 mg of $C_{19}H_{25}NO.C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Levallorphan Tartrate Injection

レバロルファン酒石酸塩注射液

Levallorphan Tartrate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$: 433.49).

Method of preparation Prepare as directed under Injection, with Levallorphan Tartrate.

Description Levallorphan Tartrate Injection is a clear, colorless liquid.

pH: 3.0 – 4.5

Identification Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate according to the labeled amount, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remained by warming on a water bath, and after cooling, add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 281 nm.

Bacterial endotoxins <*4.01*> Less than 150 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take exactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$), add exactly 10 mL of the internal

standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of levallorphan tartrate for assay, previously dried at 80°C for 4 hours on phosphorus (V) oxide under reduced pressure, and dissolve in water to make exactly 100 mL. Pipet 2 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of levallorphan to that of the internal standard:

$$\text{Amount (mg) of } C_{19}H_{25}NO.C_4H_6O_6 = M_S \times Q_T/Q_S \times 1/50$$

M_S: Amount (mg) of levallorphan tartrate for assay

Internal standard solution—Dissolve 0.04 g of isobutyl parahydroxybenzoate in 10 mL of ethanol (95), add water to make 100 mL, and to 10 mL of this solution add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 300 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of levallorphan is about 12 minutes.

System suitability—

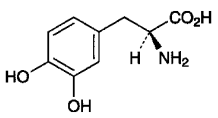
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and levallorphan are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of levallorphan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Levodopa

レボドパ



$C_9H_{11}NO_4$: 197.19
3-Hydroxy-L-tyrosine
[*59-92-7*]

Levodopa, when dried, contains not less than 98.5% of $C_9H_{11}NO_4$.

Description Levodopa occurs as white or slightly grayish white crystals or crystalline powder. It is odorless.

It is freely soluble in formic acid, slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

The pH of a saturated solution of Levodopa is between 5.0 and 6.5.

Melting point: about 275°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.

(2) To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminoantipyrine TS, and shake: a red color develops.

(3) Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (280 nm): 136 – 146 (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −11.5 – −13.0° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 nm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of Levodopa in 6 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 0.3 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Levodopa 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 <*1.11*>—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(6) Related sulstances—Dissolve 0.10 g of Levodopa in 10 mL of sodium disulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium disulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium disulfite TS 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) 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°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

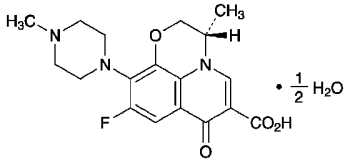
Assay Weigh accurately about 0.3 g of Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.72 mg of $C_9H_{11}NO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levofloxacin Hydrate

レボフロキサシン水和物



$C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$: 370.38
(3*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate
[*138199-71-0*]

Levofloxacin Hydrate contains not less than 99.0% and not more than 101.0% of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37), calculated on the anhydrous basis.

Description Levofloxacin Hydrate occurs as light yellowish white to yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually turns dark light yellowish white on exposure to light.

Melting point: about 226°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Levofloxacin Hydrate in 0.1 mol/L hydrochloric acid solution (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levofloxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −92 – −99° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Levofloxacin 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 substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Levofloxacin Hydrate in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 1.2 with respect to levofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of levofloxacin from the standard solution, and the area of each peak other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 1/5 times the peak area of levofloxacin from the standard solution. Furthermore, the total area of the peaks other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 3/10 times the peak area of levofloxacin from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 45°C.

Mobile phase: Dissolve 1.76 g of L-valine, 7.71 g of ammonium acetate and 1.25 g of Copper (II) sulfate pentahydrate in water to make 1000 mL. To this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of levofloxacin is about 22 minutes.

Time span of measurement: About 2 times as long as the retention time of levofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of levofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that of levofloxacin from 10 μL of the standard solution.

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 with respect to levofloxacin is not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of levofloxacin is not more than 3.0%.

(3) Residual solvent Being specified separately.

Water ⟨*2.48*⟩ 2.1 – 2.7% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

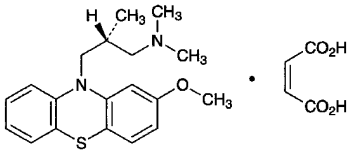
Assay Weigh accurately about 0.3 g of Levofloxacin Hydrate, dissolve in 100 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levomepromazine Maleate

レボメプロマジンマレイン酸塩



$C_{19}H_{24}N_2OS.C_4H_4O_4$: 444.54
(2*R*)-3-(2-Methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*,2-trimethylpropylamine monomaleate
[*7104-38-3*]

Levomepromazine Maleate, when dried, contains not less than 98.0% of $C_{19}H_{24}N_2OS.C_4H_4O_4$.

Description Levomepromazine Maleate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: 184 – 190°C (with decomposition).

Identification **(1)** Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow-red color is produced.

(2) To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts ⟨*2.60*⟩ between 124°C and 128°C.

(3) To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts ⟨*2.60*⟩ between 128°C and 136°C.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −13.5 – −16.5° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity **(1)** Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, 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, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Levomepromazine 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).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (2 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

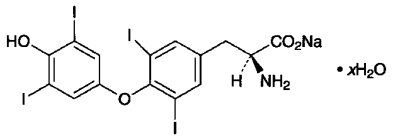
Assay Weigh accurately about 1 g of Levomepromazine Maleate, previously dried, and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.45 mg of $C_{19}H_{24}N_2OS.C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物



$C_{15}H_{10}I_4NNaO_4.xH_2O$
Monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate hydrate
[*25416-65-3*]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85), calculated on the dried basis.

Description Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insolu-

ble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification **(1)** Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

(3) Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Moisten Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests <*1.09*> (1) and (2) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −5 – −6° (0.3 g, calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. To the filtrate add water to make 10 mL, then add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

(3) Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the red-purple spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> 7 – 11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Assay Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <*2.50*> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.6657 mg of $C_{15}H_{10}I_4NNaO_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Levothyroxine Sodium Tablets

レボチロキシンナトリウム錠

Levothyroxine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85).

Method of preparation Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

Identification **(1)** Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

(2) To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a

mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R*f value.

Purity Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 25 mL of water, warm to 40°C, shake for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (%) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of the mean value of the 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9:1) (3 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel.

Column temperature: A constant temperature at about 25°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine is about 9 minutes.

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

Dissolution Being specified separately.

Assay Weigh accurately and powder not less than 20 Levothyroxine Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 3 mg of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), into a crucible, and add potassium carbonate amounting to twice the mass of the powder. In the case that the weighed powder is less than 4 g, add 8 g of potassium carbonate to the crucible. Mix well, and gently tap the crucible on the bench to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again by tapping. Heat the crucible strongly at a temperature between 675°C and 700°C for 25 minutes. Cool, add 30 mL of water, heat gently to boiling, and filter into a flask. To the residue add 30 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with hot water until the filtrate measures 300 mL. Add slowly 7 mL of freshly prepared bromine TS and diluted phosphoric acid (1 in 2) in the ratio of 3.5 mL to 1 g of the added potassium carbonate, and boil until starch-potassium iodide paper is no longer colored blue by the evolved gas. Wash the inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume of not less than 250 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse the inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate ⟨*2.50*⟩ immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.3329 mg of $C_{15}H_{10}I_4NNaO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Lidocaine

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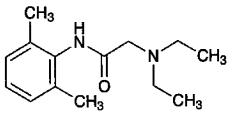

$C_{14}H_{22}N_2O$: 234.34
2-Diethylamino-*N*-(2,6-dimethylphenyl)acetamide
[*137-58-6*]

Lidocaine, when dried, contains not less than 99.0% of $C_{14}H_{22}N_2O$.

Description Lidocaine occurs as white to pale yellow crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95), soluble in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 40 mg of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩,

and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lidocaine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 66 – 69°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and 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 0.041%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and 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, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096%).

(4) Heavy metals ⟨*1.07*⟩—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed 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) Related substances—Dissolve 0.10 g of Lidocaine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.43 mg of $C_{14}H_{22}N_2O$

Containers and storage Containers—Tight containers.

Lidocaine Injection

Lidocaine Hydrochloride Injection

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Lidocaine Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O$.HCl: 270.80).

Method of preparation Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

Description Lidocaine Injection is a colorless, clear liquid.
pH: 5.0 – 7.0

Identification To a volume of Lidocaine Injection, equivalent to 20 mg of lidocaine hydrochloride ($C_{14}H_{22}N_2O$.HCl) according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 261 nm and 265 nm.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.0 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride ($C_{14}H_{22}N_2O$.HCl), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lidocaine to that of the internal standard.

Amount (mg) of lidocaine hydrochloride ($C_{14}H_{22}N_2O$.HCl)
$= M_S \times Q_T/Q_S \times 1.156$

M_S: Amount (mg) of lidocaine for assay

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 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 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of lidocaine is about 6 minutes.

System suitability—

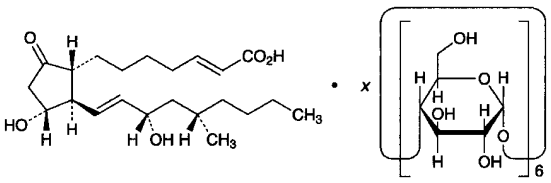
System performance: When proceed with 5 μL of the standard solution under the above operating conditions, lidocaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

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

Containers and storage Containers—Hermetic containers.

Limaprost Alfadex

リマプロスト　アルファデクス



$C_{22}H_{36}O_5 \cdot xC_{36}H_{60}O_{30}$

(2*E*)-7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*,5*S*)-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclopentyl}hept-2-enoic acid-α-cyclodextrin [*100459-01-6*, limaprost:alfadex = 1:1; clathrate compound]

Limaprost Alfadex is a α-cyclodextrin clathrate compound of limaprost.

It contains not less than 2.8% and not more than 3.2% of limaprost ($C_{22}H_{36}O_5$: 380.52), calculated on the anhydrous basis.

Description Limaprost Alfadex occurs as a white powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in ethyl acetate.

It is hygroscopic.

Identification **(1)** Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution from the sample solution (2) does not develop any color.

(2) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

(3) To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Limaprost Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it does not exhibit a maximum between 200 nm and 400 nm. To 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +125 – 135° (0.1 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

Purity Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following operating conditions, and determine each peak area by the automatic integration method: the area of the peak of 17-epi-isomer, having the relative retention time of about 1.1 with respect to limaprost, and the area of the peak of 11-deoxy substance, having the relative retention time of about 2.1, are not larger than the peak area of limaprost from the standard solution (2), and the area of the peak other than the principal peak and the peaks mentioned above is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (1).

Operating conditions—

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

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution (1) add dilute ethanol to make exactly 10 mL. Confirm that the peak area of limaprost obtained from

3 μL of this solution is equivalent to 8 to 12% of that from 3 μL of the standard solution (1).

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

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution (1) under the above conditions, the relative standard deviation of the peak area of limaprost is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Limaprost Afladex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Limaprost RS, dissolve in 5 mL of water, 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 sample solution and the standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of limaprost to that of the internal standard.

Amount (mg) of limaprost ($C_{22}H_{36}O_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Limaprost RS

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile for liquid chromatography and 2-propanol for liquid chromatography (9:5:2).

Flow rate: Adjust the flow rate so that the retention time of limaprost is about 12 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and limaprost are eluted in this order with the resolution between these peaks being not less than 7.

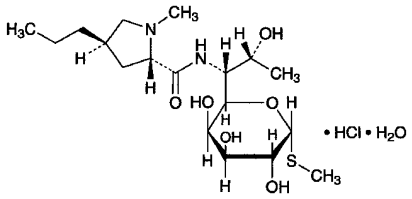
System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of limaprost to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding −10°C.

Lincomycin Hydrochloride Hydrate

リンコマイシン塩酸塩水和物



$C_{18}H_{34}N_2O_6S.HCl.H_2O$: 461.01

Methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside monohydrochloride monohydrate [*7179-49-9*]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 825 μg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Description Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification **(1)** Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 – 5.5.

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate 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) Lincomycin B—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and linco-

mycin B.

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: Measure exactly 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of lincomycin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the sample solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water ⟨*2.48*⟩ 3.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of lincomycin.

Amount [μg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Lincomycin Hydrochloride RS

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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 46°C.

Mobile phase: To 13.5 mL phosphoric acid add water to make 1000 mL, and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of lincomycin 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 number of theoretical plates and the symmetrical factor of the peak of lincomycin are not less than 4000 and not more than 1.3, respectively.

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

Containers and storage Containers—Tight containers.

Lincomycin Hydrochloride Injection

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Lincomycin Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Method of preparation Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

Description Lincomycin Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate according to the labeled amount, add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28), and add water to make 1000 mL. To 80 mL of this solution add 40 mL of 2-propanol and 90 mL of ethyl acetate, shake, develop the plate with the upper layer of this solution to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000) on the plate: the principal spots from the sample solution and standard solution show the same *R*f value.

pH ⟨*2.54*⟩ 3.5 – 5.5

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Lincomycin Hydrochloride Injection, equivalent to about 0.3 g (potency) of Lincomycin Hydrochloride Hydrate, add the mobile phase to make exactly 30 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lincomycin Hydrochloride RS, equivalent to 20 mg (potency), dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.

Amount [mg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$)
$= M_S \times A_T/A_S \times 15$

M_S: Amount [mg (potency)] of Lincomycin Hydrochloride RS

Containers and storage Containers—Hermetic containers.

Liothyronine Sodium

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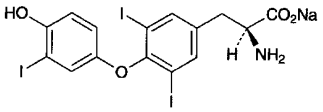

$C_{15}H_{11}I_3NNaO_4$: 672.96
Monosodium *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosinate
[*55-06-1*]

Liothyronine Sodium contains not less than 95.0% of $C_{15}H_{11}I_3NNaO_4$, calculated on the dried basis.

Description Liothyronine Sodium occurs as a white to light brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification **(1)** To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.

(2) Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

(3) Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +18 – +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

Purity **(1)** Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.

(2) Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

(3) Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia TS (1 in 3) 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 <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 4.0% (0.2 g, 105°C, 2 hours).

Assay Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <*2.50*> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.7477 mg of $C_{15}H_{11}I_3NNaO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Liothyronine Sodium Tablets

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Liothyronine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$: 672.96).

Method of preparation Prepare as directed under Tablets, with Liothyronine Sodium.

Identification **(1)** To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator, add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in methanol to make 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R*f value.

(2) The colored solution obtained in the Assay is blue in color.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L sodium hydroxide TS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

Operating conditions—

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

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted methanol (57 in 100).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability—

System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine 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 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Assay Weigh accurately not less than 20 Liothyronine Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible, and compact the charge again in the same manner. Ignite the combined mixture in the crucible between 675°C and 700°C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boiling, and filter the contents of the crucible through a glass filter (G4) into a 20-mL volumetric flask. Wash the residue with water, and combine the washings with the filtrate. Cool, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. To 2 mL of this solution, exactly measured, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use the solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into

glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS, and heat on a water bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes with occasional shaking. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared, diluted potassium iodide TS (1 in 40), swirl to mix, and transfer each solution to a 20-mL volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL, and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the sample solution as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at the wavelength of maximum absorption at about 600 nm, respectively.

Amount (mg) of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) $= M_S \times A_T/A_S \times 1/2000 \times 1.351$

M_S: Amount (mg) of potassium iodide for assay

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Lisinopril Hydrate

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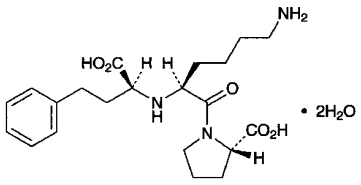

$C_{21}H_{31}N_3O_5 \cdot 2H_2O$: 441.52
(2*S*)-1-{(2*S*)-6-Amino-2-[(1*S*)-1-carboxy-3-phenylpropylamino]hexanoyl}pyrrolidine-2-carboxylic acid dihydrate
[*83915-83-7*]

Lisinopril Hydrate contains not less than 98.5% and not more than 101.0% of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49), calculated on the anhydrous basis.

Description Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Melting point: about 160°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lisinopril Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: -43.0 – $-47.0°$ (0.25 g calculated on the anhydrous basis, 0.25 ml/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Lisinopril 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 substances—Dissolve about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside 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 60°C.

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	90 → 50	10 → 50
10 – 25	50	50

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15

μL of this solution under the above operating conditions, lisinopril and caffeine 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 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.55 mg of $C_{21}H_{31}N_3O_5$

Containers and storage Containers—Well-closed containers.

Lisinopril Tablets

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Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49).

Method of preparation Prepare as directed under Tablets, with Lisinopril Hydrate.

Identification To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of lisinopril 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 ⟨*2.03*⟩. Spot 30 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120°C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their *R*f values are the same.

Purity Related substances—Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent to about 25 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of lisinopril diketopiperazine, having the relative retention time of about 2.0 with respect to lisinopril, is not larger than 2/3 times the peak area of lisinopril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Lisinopril Hydrate.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Purity (2) under Lisinopril Hydrate.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Lisinopril Tablets add exactly 5 mL each of the internal standard solution per every 1 mg of lisinopril ($C_{21}H_{31}N_3O_5$), shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$)
$= M_S \times Q_T/Q_S \times C/10$

M_S: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of a 5 mg tablet in 60 minutes and that of a 10-mg tablet in 90 minutes is not less than 80%, and that of a 20-mg tablet in 90 minutes is not less than 75%.

Start the test with 1 tablet of Lisinopril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 5.6 μg of lisinopril ($C_{21}H_{31}N_3O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay, separately determined the water ⟨*2.48*⟩ in the same manner as Lisinopril Hydrate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of lisinopril.

Dissolution rate (%) with respect to the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of lisinopril for assay, calculated on the

anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Operating conditions—

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

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

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 7 minutes.

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 lisinopril are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Lisinopril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of the internal standard solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of lisinopril for assay, separately determined the water <*2.48*> in the same manner as Lisinopril Hydrate, add exactly 50 mL of the internal standard solution to dissolve, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lisinopril to that of the internal standard.

$$\text{Amount (mg) of lisinopril } (C_{21}H_{31}N_3O_5) = M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000).

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside 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 60°C.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (19:1).

Flow rate: Adjust the flow rate so that the retention time of lisinopril 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, lisinopril and the internal standard 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lisinopril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Lithium Carbonate

炭酸リチウム

Li_2CO_3: 73.89

Lithium Carbonate, when dried, contains not less than 99.5% of Li_2CO_3.

Description Lithium Carbonate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid.

The pH of a solution dissolved 1.0 g of Lithium Carbonate in 100 mL or water is between 10.9 and 11.5.

Identification **(1)** Perform the test as directed under Flame Coloration Test <*1.04*> (1) with Lithium Carbonate: a persistent red color appears.

(2) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogen phosphate TS: a white precipitate is produced. To the precipitate add 2 mL of dilute hydrochloric acid: it dissolves.

(3) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <*1.09*> for carbonate.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to incinerate: the mass of the residue is not more than 1.5 mg.

(3) Chloride <*1.03*>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(4) Sulfate <*1.14*>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, 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 with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <*1.07*>—To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a slight red color, then add 2 mL of dilute acetic 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: Evaporate 10 mL of hydrochloric acid on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 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 5 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of Lithium Carbonate according to Method 2 using 11 mL of dilute hydrochloric acid, and perform the test according to Method B. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Aluminum—To 5.0 g of Lithium Carbonate add 20 mL of water, add gradually 15 mL of hydrochloric acid while stirring, and evaporate to dryness on a water bath. To the residue add 50 mL of water to dissolve, filter if necessary, and assign this solution as solution A. Separately, evaporate 15 mL of hydrochloric acid to dryness on a water bath, then proceed in the same manner, and assign the solution so obtained as solution B. To 10 mL of solution A add 10 mL of water and 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and shake. Add 1 mL of a solution of L-ascorbic acid (1 in 100), 2 mL of aluminon TS and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 0.1758 g of aluminum potassium sulfate dodecahydrate in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of solution B obtained in (7) and water to make 20 mL, add 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and proceed in the same manner.

(8) Barium—To 20 mL of solution A obtained in (7) add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethanol (95) and 2 mL of potassium sulfate TS, and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 17.8 mg of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution add 20 mL of solution B obtained in (7), 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol (95), and proceed in the same manner.

(9) Calcium—Weigh accurately about 5 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid, and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS, and allow to stand for 4 hours. Filter the produced precipitate through a glass filter (G4), wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70°C and 80°C, and titrate with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (Ca: 40.08) is not more than 0.05%.

Each mL of 0.02 mol/L potassium permanganate VS
= 2.004 mg of Ca

(10) Magnesium—To 3.0 mL of solution A obtained in (7) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(11) Potassium—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

(12) Sodium—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity L_S shows 100 adjustment, and determine emission intensity L_T of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity L_B of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

$$\text{Amount (\%) of sodium (Na)} = (L_T - L_B)/(L_S - L_T) \times M'/M \times 100$$

M: Amount (mg) of the sample in 25 mL of the sample stock solution

M': Amount (mg) of sodium in 20 mL of the standard solution

(13) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

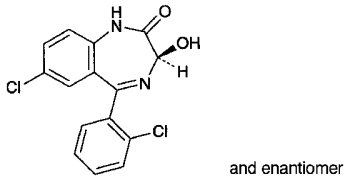
Assay Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate <2.50> the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS
= 36.95 mg of Li_2CO_3

Containers and storage Containers—Well-closed containers.

Lorazepam

ロラゼパム



and enantiomer

$C_{15}H_{10}Cl_2N_2O_2$: 321.16
(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*846-49-1*]

Lorazepam, when dried, contains not less than 98.5% of $C_{15}H_{10}Cl_2N_2O_2$.

Description Lorazepam occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Identification **(1)** To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Determine the absorption spectrum of a solution of Lorazepam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Lorazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Lorazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Absorbance <2.24> $E^{1\%}_{1cm}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

Purity **(1)** Chloride <1.03>—To 1.0 g of Lorazepam 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%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Lorazepam 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 <1.11>—Prepare the test solution with 1.0 g of Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 <2.03>. Spot 10 μL each of the sample 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, 1,4-dioxane and acetic acid (100) (91:5:4) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

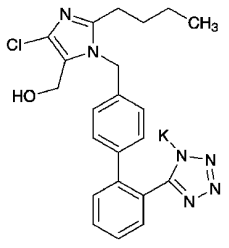
Assay Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate <2.50> with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
= 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Losartan Potassium

ロサルタンカリウム



$C_{22}H_{22}ClKN_6O$: 461.00
Monopotassium 5-{[4′-(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl}-1*H*-tetrazol-1-ide
[*124750-99-8*]

Losartan Potassium contains not less than 98.5% and not more than 101.0% of $C_{22}H_{22}ClKN_6O$, calcu-

lated on the anhydrous basis.

Description Losartan Potassium occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Losartan Potassium in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Losartan Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Losartan Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Losartan Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Losartan Potassium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Losartan Potassium 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 30 mg of Losartan Potassium in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of solvent and losartan obtained from the sample solution is not larger than 1/10 times the peak area of losartan from the standard solution, and the total area of the peaks other than the peak of losartan from the sample solution is not larger than 3/10 times the peak area of losartan from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside 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: Diluted phosphoric acid (1 in 1000).

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 25	75 → 10	25 → 90
25 – 35	10	90

Flow rate: 1.0 mL per minute.

Time span of measurement: 35 minutes after injection of the sample.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 to 13% of that of losartan 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 the symmetry factor of the peak of losartan are not less than 10,000 and not more than 1.3, respectively.

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

(3) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ Not more than 0.5% (0.25 g, volumetric titration, direct titration).

Assay Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Losartan Potassium), dissolve separately in methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

$$\text{Amount (mg) of losartan potassium } (C_{22}H_{22}ClKN_6O) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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 35°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of losartan is about 6 minutes.

System suitability—

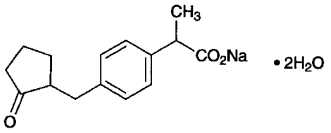
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of losartan are not less than 5500 and not more than 1.4, respectively.

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

Containers and storage Containers—Tight containers.

Loxoprofen Sodium Hydrate

ロキソプロフェンナトリウム水和物



$C_{15}H_{17}NaO_3.2H_2O$: 304.31
Monosodium 2-{4-[(2-oxocyclopentyl)methyl]phenyl}propanoate dihydrate
[*80382-23-6*]

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28), calculated on the anhydrous basis.

Description Loxoprofen Sodium Hydrate occurs as white to yellowish white crystals or crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

Identification **(1)** Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid for Color A (1 in 2).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Loxoprofen 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).

(3) Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane and acetic acid (100) (9:1) 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 sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 11.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Loxoprofen RS, previously dried in a desiccator (in vacuum, 60°C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the sample solution, and use so obtained solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of loxoprofen to that of the internal standard.

$$\text{Amount (mg) of loxoprofen sodium } (C_{15}H_{17}NaO_3) = M_S \times Q_T/Q_S \times 1.089$$

M_S: Amount (mg) of Loxoprofen RS

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (7 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 4.6 mm in inside 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, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

System suitability—

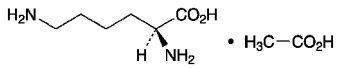
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Lysine Acetate

L-リシン酢酸塩



$C_6H_{14}N_2O_2.C_2H_4O_2$: 206.24
(2*S*)-2,6-Diaminohexanoic acid monoacetate
[*57282-49-2*]

L-Lysine Acetate, when dried, contains not less than 98.5% and not more than 101.0% of $C_6H_{14}N_2O_2.C_2H_4O_2$.

Description L-Lysine Acetate occurs as white crystals or crystalline powder. It has a characteristic odor and a slightly acid taste.

It is very soluble in water, freely soluble in formic acid, and practically insoluble in ethanol (99.5).

It is deliquescent.

Identification **(1)** Determine the infrared absorption spectrum of L-Lysine Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Lysine Acetate (1 in 20) responds to the Qualitative Tests <*1.09*> (2) for acetate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +8.5 – +10.0° (after drying, 2.5 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

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

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Lysine Acetate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Lysine Acetate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Lysine Acetate. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Lysine Acetate 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).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Lysine Acetate according to Method 1, 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—Weigh accurately about 0.5 g of L-Lysine Acetate, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately 2.5 mol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Based on the peak heights of the amino acids obtained from the sample solution and standard solution, determine the mass of the amino acids other than lysine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acids other than lysine is not more than 0.1%.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and to each phase add 0.1 mL of capric acid.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20 mL	4 mL	—	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	—	—
Benzyl alcohol	—	—	—	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing mobile phases: Proceed with 20 μL of the standard solution under the above operating conditions: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order. Switchover the mobile phases A, B, C, D and E in sequence so that the resolution between the peaks of isoleucine and leucine is not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, and add 123 mL of acetic acid (100), 401 mL

of 1-methoxy-2-propanol, and water to make 1000 mL, gas with nitrogen for 10 minutes, and use this solution as the solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, gas with nitrogen for 5 minutes, add 81 mg of sodium borohydride, gas the solution with nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of the solution (I) add 1 volume of the solution (II). Prepare before use.

Mobile phase flow rate: 0.20 mL per minute.

Reaction reagent flow rate: 0.24 mL per 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 glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Acetate, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

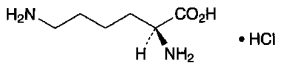
Each mL of 0.1 mol/L perchloric acid VS
= 10.31 mg of $C_6H_{14}N_2O_2.C_2H_4O_2$

Containers and storage Containers—Tight containers.

L-Lysine Hydrochloride

Lysine Hydrochloride

L-リシン塩酸塩



$C_6H_{14}N_2O_2.HCl$: 182.65
(2*S*)-2,6-Diaminohexanoic acid monohydrochloride
[*657-27-2*]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of $C_6H_{14}N_2O_2.HCl$.

Description L-Lysine Hydrochloride occurs as a white powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

Identification **(1)** Determine the infrared absorption spectrum of L-Lysine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

(2) A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D$: +19.0 – +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of L-Lysine Hydrochloride in 10 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 L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of L-Lysine 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).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, pipet 5 mL of this 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 ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia water (28) (67:33) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

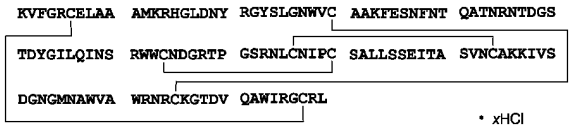
Assay Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate ⟨*2.50*⟩ the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.132 mg of $C_6H_{14}N_2O_2.HCl$

Containers and storage Containers—Tight containers.

Lysozyme Hydrochloride

リゾチーム塩酸塩



$C_{616}H_{963}N_{193}O_{182}S_{10}.x$HCl
[*12650-88-3*, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

Description Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

Identification **(1)** To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Lysozyme 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).

Loss on drying <*2.41*> Not more than 8.0% (0.1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 2.0% (0.5 g).

Nitrogen Perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is between 16.8% and 18.6%, calculated on the dried basis.

Assay Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lysozyme RS (separately determine its loss on drying <*2.41*> in the same manner as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution, pH 6.2 to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 μL of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry <*2.24*>, A_T, of this solution at 640 nm, using water as the blank. Determine the absorbances, A_{S1} and A_{S2}, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis
$= M_S/2M_T \times \{(A_{S1} - A_T)/(A_{S1} - A_{S2}) + 1\}$

M_S: Amount (mg) of Lysozyme RS, calculated on the dried basis.

M_T: Amount (mg) of the sample, calculated on the dried basis.

Containers and storage Containers—Tight containers.

Macrogol 400

Polyethylene Glycol 400

マクロゴール **400**

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 7 to 9.

Description Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4 – 8°C

Specific gravity d_{20}^{20}: 1.110 – 1.140

Identification Dissolve 50 mg of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH <*2.54*> Dissolve 1.0 g of Macrogol 400 in 20 mL of water: the pH of this solution is between 4.0 and 7.0.

Purity **(1)** Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogol 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve

in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨2.02⟩ according to the following conditions. Determine the peak heights, H_{Ta} and H_{Sa}, of ethylene glycol of each solution, and the peak heights, H_{Tb} and H_{Sb}, of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

$$\text{Amount (mg) of ethylene glycol} = M_{Sa} \times H_{Ta}/H_{Sa} \times 1/10$$

$$\text{Amount (mg) of diethylene glycol} = M_{Sb} \times H_{Tb}/H_{Sb} \times 1/10$$

M_{Sa}: Amount (mg) of ethylene glycol for gas chromatography

M_{Sb}: Amount (mg) of diethylene glycol for gas chromatography

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A colum about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μm in particle diameter, coated with D-sorbitol at the ratio of 12%.

Column temperature: A constant temperature of about 165°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of diethylene glycol is about 8 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 μL of the standard solution composes about 80% of the full scale.

Average molecular mass Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolved the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogol 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate ⟨2.50⟩ with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of sample

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 380 and 420.

Water ⟨2.48⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Macrogol 1500

Polyethylene Glycol 1500

マクロゴール **1500**

Macrogol 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

Description Macrogol 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 – 41°C

Identification Dissolve 50 mg of Macrogol 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH ⟨2.54⟩ Dissolve 1.0 g of Macrogol 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 1500 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(3) Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogol 1500 in a distilling flask, add 75 mL of diphenyl ether, warm to dissolve if necessary, distil slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diphenyl ether, and filtrate into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sam-

ple solution and the standard solution, and add to each exactly 15 mL of cerium (IV) diammonium nitrate TS. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ within 2 to 5 minutes: the absorbance of the solution obtained from the sample solution at the wavelength of maximum absorption at about 450 nm is not larger than the absorbance of the solution obtained from the standard solution.

Water ⟨*2.48*⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Macrogol 4000

Polyethylene Glycol 4000

マクロゴール **4000**

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 59 to 84.

Description Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

Congealing point: 53 – 57°C

Identification Dissolve 50 mg of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

Purity **(1)** Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthelein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate ⟨*2.50*⟩ with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 2600 and 3800.

Water ⟨*2.48*⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol 6000

Polyethylene Glycol 6000

マクロゴール **6000**

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 165 to 210.

Description Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is ordorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

Congealing point: 56 – 61°C

Identification Dissolve 50 mg of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 6000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dis-

solve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate ⟨*2.50*⟩ with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 7300 and 9300.

Water ⟨*2.48*⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol 20000

Polyethylene Glycol 20000

マクロゴール **20000**

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of *n* lies between 340 and 570.

Description Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is ordorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in dehydrated diethyl ether, in petroleum benzine and in macrogol 400.

Congealing point: 56 – 64°C

Identification Dissolve 50 mg of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at 98 ± 2°C for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate ⟨*2.50*⟩ with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 15000 and 25000.

Water ⟨*2.48*⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol Ointment

Polyethylene Glycol Ointment

マクロゴール軟膏

Method of preparation

Macrogol 4000	500 g
Macrogol 400	500 g
To make	1000 g

Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C, and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

Description Macrogol Ointment is white in color. It has a faint, characteristic odor.

Identification Dissolve 50 mg of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.

Containers and storage Containers—Tight containers.

Magnesium Carbonate

炭酸マグネシウム

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate.

Magnesium Carbonate contains not less than 40.0% and not more then 44.0% of magnesium oxide (MgO: 40.30).

''Heavy magnesium carbonate'' may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

Description Magnesium Carbonate occurs as white, friable masses or powder. It is odorless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in 1-propanol.

It dissolves in dilute hydrochloric acid with effervescence.

Its saturated solution is alkaline.

Identification **(1)** Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt.

(2) Magnesium Carbonate responds to the Qualitative Tests <*1.09*> (1) for carbonate.

Purity **(1)** Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue does not exceed 10.0 mg.

(2) Heavy metals <*1.07*>—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).

(3) Iron <*1.10*>—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).

(5) Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

Precipitation test Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in ml).

Assay Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction. From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

Containers and storage Containers—Well-closed containers.

Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of MgO.

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

Description Magnesium Oxide occurs as a white powder or granules. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

It absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests <*1.09*> for magnesium salt.

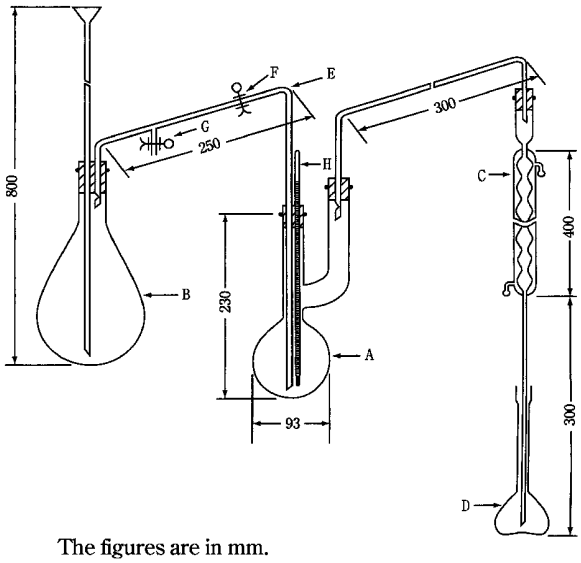
Purity **(1)** Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

(2) Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.



The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling tips 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 clamp
H: Thermometer

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

(6) Arsenic <*1.11*>—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

(7) Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

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

(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 wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed 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°C, then open the rub-

ber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. 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 Oxygen Flask Combustion Method ⟨*1.06*⟩. No corrective solution is used in this procedure. The content of fluoride (F) is not more than 0.08%.

Amount (mg) of fluoride (F: 19.00) in the test solution
= amount (mg) of fluoride in 5 mL of the standard solution
× A_T/A_S × 200/V

Loss on ignition ⟨*2.43*⟩ Not more than 10% (0.25 g, 900°C, constant mass).

Assay Ignite Magnesium Oxide to constant mass at 900°C, weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed, deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

Containers and storage Containers—Tight containers.

Magnesium Silicate

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide (SiO_2: 60.08) and not less than 20.0% of magnesium oxide (MgO: 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

Description Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification **(1)** Mix 0.5 g of Magnesium Silicate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for magnesium salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate, and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

Purity **(1)** Soluble salts—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water bath for 60 minutes with occasional shaking, then cool, dilute with water to 150 mL, and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 0.02 g.

(2) Alkalinity—To 20 mL of the sample solution obtained in (1) add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Chloride ⟨*1.03*⟩—Take 10 mL of the sample solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate ⟨*1.14*⟩—To the residue obtained in (1) add about 3 mL of dilute hydrochloric acid, and heat on a water bath for 10 minutes. Add 30 mL of water, filter, wash the residue on the filter with water, combine the washings with the filtrate, and dilute to 50 mL with water. To 4 mL of the solution 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 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals ⟨*1.07*⟩—To 1.0 g of Magnesium Silicate add 20 mL of water and 3 mL of hydrochloric acid, and boil for 2 minutes. Filter, and wash the residue on the filter with two 5-mL portions of water. Evaporate the combined filtrate and washings on a water bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until solution is complete, filter, if necessary, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(6) Arsenic ⟨*1.11*⟩—To 0.4 g of Magnesium Silicate add 5 mL of dilute hydrochloric acid, heat gently to boiling 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 on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on ignition ⟨*2.43*⟩ Not more than 34% (0.5 g, 850°C, 3 hours).

Acid-consuming capacity ⟨*6.04*⟩ Place about 0.2 g of Magnesium Silicate, accurately weighed, in a glass-stoppered flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2°C for 1 hour, and cool. Pipet 25 mL of the supernatant liquid, and titrate ⟨*2.50*⟩ the excess hydrochloric acid, while stirring well, with

0.1 mol/L sodium hydroxide VS until the pH becomes 3.5.

1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

Assay **(1)** Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto the filter paper, and wash with hot water until the last washing does not respond to the Qualitative Tests <*1.09*> (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating, and ignite between 775°C and 825°C for 30 minutes, then cool, and weigh the residue as a (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as b (g).

$$\text{Content (\%) of silicon dioxide } (SiO_2) = (a - b)/M \times 100$$

M: Mass (g) of the sample

(2) Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2,2′,2″-nitrilotrisethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

$$\text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 2.015 \text{ mg of MgO}$$

(3) Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide (SiO_2)—Calculate the quotient from the percentages obtained in (1) and (2).

Containers and storage Containers—Well-closed containers.

Magnesium Stearate

ステアリン酸マグネシウム

Magnesium Stearate consists chiefly magnesium salts of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

It contains, when dried, not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31).

Description Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

Identification **(1)** Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make exactly 50 mL, mix, and use this solution as the sample solution: the sample solution responds to the Qualitative Tests <*1.09*> for magnesium.

(2) The retention times of the peaks corresponding to methyl stearate and methyl palmitate in the chromatogram of the sample solution correspond to those of methyl stearate and methyl palmitate in the chromatogram of the system suitability solution, as obtained in the Purity (5).

Purity **(1)** Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, and filter after cooling. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS, and add exactly 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS: the color of the solution changes.

(2) Chloride <*1.03*>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 1.40 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.10%).

(3) Sulfate <*1.14*>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 10.2 mL of 0.01 mol/L sulfuric acid VS (not more than 1.0%).

(4) Heavy metals <*1.07*>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(5) Relative content of stearic acid and palmitic acid—Transfer exactly 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for about 10 minutes to dissolve the solids. Add 4.0 mL of heptane through the condenser, and reflux for about 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, to another

flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, mix, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography <*2.02*> according to the following conditions, and determine the area, A, of the methyl stearate peak and the total of the areas, B, of all of fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

$$\text{Content (\%) of stearic acid} = A/B \times 100$$

Similarly, calculate the percentage of palmitic acid in Magnesium Stearate. The methyl stearate peak, and the total of the methyl stearate and methyl palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively, in the chromatogram.

Operating conditions—

Detector: A hydrogen flame-ionization detector maintained at a constant temperature of about 260°C.

Sample injection port: A splitless injection system maintained at a constant temperature of about 220°C.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain this temperature for 5 minutes.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of methyl stearate is about 32 minutes.

Split ratio: Splitless.

Time span of measurement: About 1.5 time as long as the retention time of methyl stearate beginning after the solvent peak.

System suitability—

Test for required detection: Place exactly 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography, each previously dried in a desiccator (silica gel) for 4 hours, in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. To exactly 1 mL of the solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 5 to 15% of that from 1 μL of the solution for system suitability test.

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, methyl palmitate and methyl stearate are eluted in this order, with the relative retention time of methyl palmitate to methyl stearate being about 0.86, and with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 6.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

Loss on drying <*2.41*> Not more than 6.0% (2 g, 105°C, constant mass).

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 5×10^2 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.

Assay Transfer about 0.5 g of previously dried Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of 1-butanol and ethanol (99.5) (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45°C to 50°C to make the solution clear, and after cooling, titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to purple in color. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.431 mg of Mg

Containers and storage Containers—Tight containers.

Magnesium Sulfate Hydrate

硫酸マグネシウム水和物

$MgSO_4.7H_2O$: 246.47

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate ($MgSO_4$: 120.37).

Description Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste.

It is very soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests <*1.09*> for magnesium salt and for sulfate.

pH <*2.54*> Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

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

(2) Chloride <*1.03*>—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Magnesium Sulfate 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) Zinc—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.

(5) Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of Standard Calcium Solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the absorbances, A_T and A_S, of both solutions: A_T is not bigger than $A_S - A_T$ (not more than 0.02%).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Calcium hollow-cathod lamp.
Wavelength: 422.7 nm.

(6) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on ignition <*2.43*> 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (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 dihydrogen ethylenediamine tetraacetate VS
= 6.018 mg of $MgSO_4$

Containers and storage Containers—Well-closed containers.

Magnesium Sulfate Injection

硫酸マグネシウム注射液

Magnesium Sulfate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

Method of preparation Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

Description Magnesium Sulfate Injection is a clear, colorless liquid.

Identification Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount, and add water to make 20 mL: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt and for sulfate.

pH <*2.54*> 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

Bacterial endotoxins <*4.01*> Less than 0.09 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate ($MgSO_4.7H_2O$), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Magnesium Sulfate Mixture

硫酸マグネシウム水

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

Method of preparation

Magnesium Sulfate Hydrate	150 g
Bitter Tincture	20 mL
Dilute Hydrochloric Acid	5 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare before use, with the above ingredients.

Description Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

Identification **(1)** Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> for magnesium salt.

(2) Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> (2) for chloride.

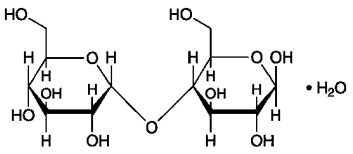
Assay Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of pH 10.7 ammonia-ammonium chloride buffer solution, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

Containers and storage Containers—Tight containers.

Maltose Hydrate

マルトース水和物



$C_{12}H_{22}O_{11}.H_2O$: 360.31
α-D-Glucopyranosyl-(1→4)-β-D-glucopyranose monohydrate
[*6363-53-7*]

Maltose Hydrate, when dried, contains not less than 98.0% of $C_{12}H_{22}O_{11}.H_2O$.

Description Maltose Hydrate occurs as white crystals or crystalline powder.

It has a sweet taste.

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

Identification **(1)** Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.

(2) Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is formed.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +126 – +131° Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

pH ⟨*2.54*⟩ The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60°C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

Control solution: Add water to a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

(2) Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(5) Arsenic ⟨*1.11*⟩—Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, then heat to concentrate to 5 mL, and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

(6) Dextrin, soluble starch and sulfite—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

(7) Nitrogen—Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩ using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

(8) Related substances—Dissolve 0.5 g of Maltose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the sample solution is not larger than 1.5 times the peak area of maltose from the standard solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 times the peak area of maltose from 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 sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.

Time span of measurement: About 2 times as long as the retention time of maltose.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose RS, previously dried, dissolve in exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of maltose to that of the internal standard.

$$\text{Amount (mg) of } C_{12}H_{22}O_{11}.H_2O = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Maltose RS

Internal standard solution—A solution of ethylene glycol (1 in 50).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8 %) (10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time

of maltose is about 18 minutes.

Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and storage Containers—Tight containers.

Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use.

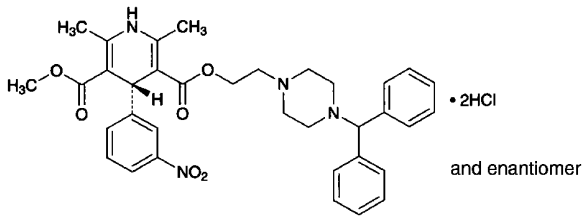
It contains *Agkistrodon Halys* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

Manidipine Hydrochloride

マニジピン塩酸塩



$C_{35}H_{38}N_4O_6.2HCl$: 683.62

3-{2-[4-(Diphenylmethyl)piperazin-1-yl]ethyl} 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride

[*126229-12-7*]

Manidipine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{35}H_{38}N_4O_6.2HCl$.

Description Manidipine Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in dimethylsulfoxide, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Manidipine Hydrochloride in dimethylsulfoxide (1 in 100) shows no optical rotation.

Manidipine Hydrochloride turns slightly brown-yellowish white on exposure to light.

Melting point: about 207°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Manidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Manidipine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Manidipine Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Manidipine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 10 mL of water to 0.1 g of Manidipine Hydrochloride, shake vigorously, and filter. Add 1 drop of ammonia TS to 3 mL of the filtrate, allow to stand 5 minutes, and filter. The filtrate responds to the Qualitative Tests <*1.09*> (2) for chlorides.

Purity **(1)** Heavy metals <*1.07*>— Proceed with 1.0 g of Manidipine 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 <*1.11*>—Prepare the test solution with 2.0 g of Manidipine Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 20 mg of Manidipine Hydrochloride in 200 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than manidipine obtained from the sample solution is not larger than 1/5 times the manidipine peak area from the standard solution. Furthermore, the total of the areas of all peaks other than manidipine is not larger than 7/10 times the peak area of manidipine 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.

Time span of measurement: About 3.5 times as long as the retention time of manidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of manidipine obtained from 20 μL of this solution is equivalent to 8 to 12% of that from 20 μL of the standard solution.

System performance: Dissolve 50 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 50 mL. To 10 mL of this solution add 5 mL of a solution of butyl benzoate in acetonitrile (7 in 5000) and the 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, manidipine and butyl benzoate

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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Manidipine Hydrochloride, previously dried, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of manidipine to that of the internal standard.

$$\text{Amount (mg) of } C_{35}H_{38}N_4O_6.2HCl = M_S \times Q_T/Q_S \times 4$$

M_S: Amount (mg) of Manidipine Hydrochloride RS

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside 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: Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 4.6 with diluted potassium hydroxide TS (1 in 10). To 490 mL of this solution add 510 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of manidipine 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, manidipine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of manidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Manidipine Hydrochloride Tablets

マニジピン塩酸塩錠

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6.2HCl$: 683.62).

Method of preparation Prepare as directed under Tablets, with Manidipine Hydrochloride.

Identification To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample 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 and diethylamine (200:1) 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 sample solution and the spot obtained from the standard solution show the same *Rf* value.

Uniformity of dosage units ⟨*6.02*⟩ 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 Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6.2HCl$), disintegrate by adding a mixture of water and acetonitrile (1:1) to make V mL so that each mL contains about 0.1 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6.2HCl$), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of manidipine hydrochloride } (C_{35}H_{38}N_4O_6.2HCl) = M_S \times Q_T/Q_S \times V/250$$

M_S: Amount (mg) of Manidipine Hydrochloride RS

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 45 minutes of Manidipine Hydrochloride Tablets is not less than 75%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Manidipine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, and add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl) according to the labeled amount. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the manidipine peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Manidipine Hydrochloride RS
C: Labeled amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside 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 acetonitrile and a solution of potassium dihydrogen phosphate (681 in 100,000) (3:2).

Flow rate: Adjust the flow rate so that the retention time of manidipine 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, the number of theoretical plates and the symmetry factor of the peak of manidipine are not less than 1500 and not more than 1.5, respectively.

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

Assay Conduct this procedure using light-resistant vessels. Weigh accurately not less than 20 Manidipine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (1:1) to make 100 mL, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make 50 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Manidipine Hydrochloride.

Amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl)
$= M_S \times Q_T/Q_S \times 2/5$

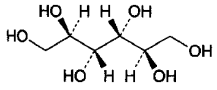
M_S: Amount (mg) of Manidipine Hydrochloride RS

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

D-Mannitol

D-マンニトール



$C_6H_{14}O_6$: 182.17
D-Mannitol
[*69-65-8*]

D-Mannitol, when dried, contains not less than 98.0% of $C_6H_{14}O_6$.

Description D-Mannitol occurs as white crystals or powder. It is odorless, and has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** To 5 drops of a saturated solution of D-Mannitol add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

(2) Determine the infrared absorption spectrum of D-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of D-Mannitol in 3 mL of warm water, then allow to stand at 5°C for 24 hours or until crystals appear, and filter. Wash the crystals so obtained with a few amount of cold water, dry at 105°C for 4 hours, and perform the test with the crystals.

Optical rotation <2.49> $[\alpha]_D^{20}$: +137 – +145° Weigh accurately about 1.0 g of D-Mannitol, previously dried, dissolve in 80 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 20), and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

Melting point <2.60> 166 – 169°C

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of D-Mannitol in 10 mL of water by warming: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of D-Mannitol in 50 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <*1.03*>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(4) Sulfate <*1.14*>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(5) Heavy metals <*1.07*>—Proceed with 5.0 g of D-Mannitol 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).

(6) Nickel—Dissolve 0.5 g of D-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(7) Arsenic <*1.11*>—Prepare the test solution with 1.5 g of D-Mannitol according to Method 1, and perform the test (not more than 1.3 ppm).

(8) Sugars—To 5.0 g of D-Mannitol add 15 mL of water and 4.0 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS), and add water to make 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling's TS for 3 minutes, and allow to stand to precipitate copper (I) oxide. Filter the supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction, and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask, filter through the glass filter described above, and wash the filter with water. Combine the washings and the filtrate, heat to 80°C, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS: the consumed volume is not more than 1.0 mL.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of D-Mannitol, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Tight containers.

D-Mannitol Injection

D-Mannite Injection

D-マンニトール注射液

D-Mannitol Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of D-mannitol ($C_6H_{14}O_6$: 182.17).

Method of preparation Prepare as directed under Injections, with D-Mannitol. No preservative is added.

Description D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste.

It may precipitate crystals.

Identification Concentrate D-Mannitol Injection on a water bath to make a saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under D-Mannitol.

pH <*2.54*> 4.5 – 7.0

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of D-Mannitol Injection, equivalent to about 5 g of D-mannitol ($C_6H_{14}O_6$), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under D-Mannitol.

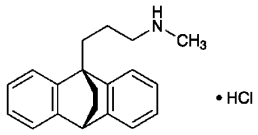
Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Maprotiline Hydrochloride

マプロチリン塩酸塩



$C_{20}H_{23}N.HCl$: 313.86
3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-*N*-methylpropylamine monohydrochloride
[*10347-81-6*]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of $C_{20}H_{23}N.HCl$.

Description Maprotiline Hydrochloride occurs as a white crystalline powder.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.

Melting point: about 244°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Maprotiline Hydrochloride in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Maprotiline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Maprotiline Hydrochloride with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.

(3) To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Maprotiline 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—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), and titrate <*2.50*> 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
= 31.39 mg of $C_{20}H_{23}N.HCl$

Containers and storage Containers—Well-closed containers.

Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒生麻しんワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use.

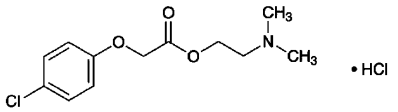
It contains live attenuated measles virus.

It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩



$C_{12}H_{16}ClNO_3.HCl$: 294.17
2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride
[*3685-84-5*]

Meclofenoxate Hydrochloride contains not less than 98.0% of $C_{12}H_{16}ClNO_3.HCl$, calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride occurs as white crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.

It is freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

Identification **(1)** To 10 mg of Meclofenoxate Hydrochlo-

ride add 2 mL of ethanol (95), dissolve by warming if necessary, cool, add 2 drops of a saturated solution of hydroxylammonium chloride in ethanol (95) and 2 drops of a saturated solution of potassium hydroxide in ethanol (95), and heat in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (III) chloride TS: a red-purple to dark purple color develops.

(2) Dissolve 50 mg of Meclofenoxate Hydrochloride in 5 mL of water, and add 2 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Meclofenoxate Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 139 – 143°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Meclofenoxate 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 <*1.11*>—Prepare the test solution with 1.0 g of Meclofenoxate Hydrochloride according to method 3, and perform the test (not more than 2 ppm).

(5) Organic acids—To 2.0 g of Meclofenoxate Hydrochloride add 50 mL of diethyl ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5-mL portions of diethyl ether, and combine the washings with the filtrate. To this solution add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS, and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

Water <*2.48*> Not more than 0.50% (1 g, volumetric titration, dirct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

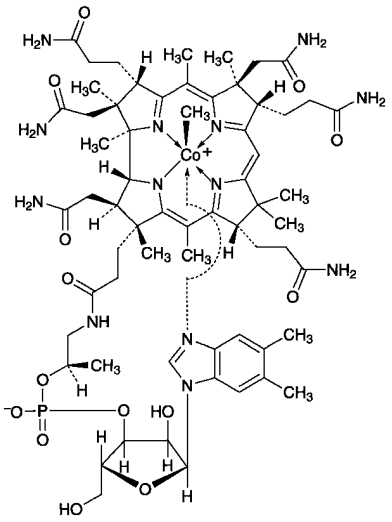
Assay Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)]. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.42 mg of $C_{12}H_{16}ClNO_3.HCl$

Containers and storage Containers—Tight containers.

Mecobalamin

メコバラミン



$C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38
*Co*α-[α-(5,6-Dimethyl-1*H*-benzoimidazol-1-yl)]-*Co*β-methylcobamide
[*13422-55-4*]

Mecobalamin contains not less than 98.0% of $C_{63}H_{91}CoN_{13}O_{14}P$, calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is affected by light.

Identification **(1)** Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Mecobalamin 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 dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 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-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

Purity **(1)** Clarity and color of solution—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red color.

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area of mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than mecobalamin is not more than 0.5% of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin 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.

Time span of measurement: About 2.5 times as long as the retention time of mecobalamin.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add the mobile phase to make exactly 10 mL. Confirm that the peak area of mecobalamin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0%.

Water <2.48> Not more than 12% (0.1 g, volumetric titration, direct titration).

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin RS (separately, determine the water <2.48> in the same manner as Mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

$$\text{Amount (mg) of } C_{63}H_{91}CoN_{13}O_{14}P = M_S \times A_T/A_S$$

M_S: Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of mecobalamin is about 12 minutes.

System suitability—

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And 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 mecobalamin is not less than 6000.

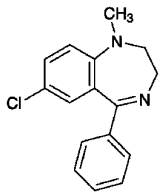
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Medazepam

メダゼパム



$C_{16}H_{15}ClN_2$: 270.76

7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine

[*2898-12-6*]

Medazepam, when dried, contains not less than 98.5% and not more than 101.0% of $C_{16}H_{15}ClN_2$.

Description Medazepam occurs as white to light yellow crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (99.5), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually turns yellow on exposure to light.

Identification **(1)** Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Medazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave

numbers.

(4) Perform the test with Medazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 101 – 104°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

(2) Chloride <*1.03*>—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, 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 0.018%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Medazepam 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 <*1.11*>—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent 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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> 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
= 27.08 mg of $C_{16}H_{15}ClN_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Medicinal Carbon

薬用炭

Description Medicinal Carbon occurs as a black, odorless and tasteless powder.

Identification Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

Purity **(1)** Acidity or alkalinity—Boil 3.0 g of Medicinal Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

(2) Chloride <*1.03*>—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(3) Sulfate <*1.14*>—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and 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 0.192%).

(4) Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of L-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distil to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

(7) Heavy metals <*1.07*>—Proceed with 0.5 g of Medicinal Carbon 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).

(8) Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL,

add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

(9) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 15.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 4.0% (1 g).

Adsorptive power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <*2.50*> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS. The difference between the two titrations is not less than 1.2 mL.

Containers and storage Containers—Well-closed containers.

Medicinal Soap

薬用石ケン

Medicinal Soap is sodium salts of fatty acids.

Description Medicinal Soap occurs as white to light yellow powder or granules. It has a characteristic odor free from rancidity.

Medicinal Soap is sparingly soluble in water, and slightly soluble in ethanol (95).

A solution of Medicinal Soap (1 in 100) is alkaline.

Fatty acid Dissolve 25 g of Medicinal Soap in 300 mL of hot water, add 60 mL of dilute sulfuric acid slowly, and warm in a water bath for 20 minutes. After cooling, filter off the precipitate, and wash with warm water until the washing no longer shows acidity to methyl orange TS. Transfer the precipitate to a small beaker, and heat on a water bath to complete separation of water and transparent fatty acids. Filter the fatty acid into a small beaker while warm, dry at 100°C for 20 minutes, and perform the test with this material as directed under Fats and Fatty Oils <*1.13*>. The congealing point of the fatty acid is between 18°C and 28°C. The acid value is 185 – 205. The iodine value is 82 – 92.

Purity (1) Acidity or alkalinity—Dissolve 5.0 g of Medicinal Soap in 85 mL of neutralized ethanol by warming on a water bath, filter while hot through absorbent cotton, and wash the filter and the residue with three 5-mL portions of hot neutralized ethanol. Combine the filtrate and the washings, add hot neutralized ethanol to make exactly 100 mL, and perform the following tests quickly using this as the sample solution at 70°C.

(i) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS to 40 mL of the sample solution: a red color develops.

(ii) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS to 40 mL of the sample solution: no red color develops.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Medicinal Soap 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) Ethanol-insoluble substances—Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

(4) Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

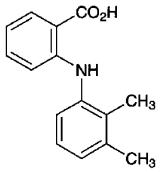
Loss on drying Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

Containers and storage Containers—Well-closed containers.

Mefenamic Acid

メフェナム酸



$C_{15}H_{15}NO_2$: 241.29
2-(2,3-Dimethylphenylamino)benzoic acid
[*61-68-7*]

Mefenamic Acid, when dried, contains not less than 99.0% of $C_{15}H_{15}NO_2$.

Description Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 225°C (with decomposition).

Identification **(1)** Dissolve 10 mg of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of *p*-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

(2) Dissolve 10 mg of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

(3) Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Chloride <*1.03*>—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the produced precipitate by filtration, discard the first 10 mL of the filtrate, and to subsequent 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 as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Mefenamic 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).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) 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 <*2.03*>. Spot 25 μL each of the sample 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-methyl-1-propanol and ammonia solution (28) (3:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

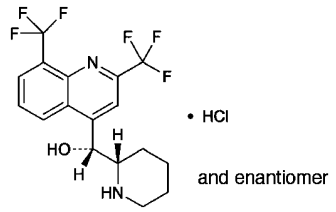
Assay Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.13 mg of $C_{15}H_{15}NO_2$

Containers and storage Containers—Well-closed containers.

Mefloquine Hydrochloride

メフロキン塩酸塩



$C_{17}H_{16}F_6N_2O.HCl$: 414.77

(1*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol monohydrochloride

[*51773-92-3*]

Mefloquine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{16}F_6N_2O.HCl$.

Description Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in sulfuric acid.

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

Melting point: about 260°C (with decomposition).

Identification **(1)** Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of

ammonia TS.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Mefloquine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than mefloquine and the peak eluted first from the sample solution is not larger than the peak area of mefloquine from the standard solution, and the total area of the peaks other than the peak of mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 14) (24:1).

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

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

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of mefloquine obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, diprophylline and mefloquine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mefloquine is not more than 2.0%.

(4) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

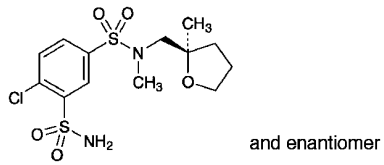
Assay Weigh accurately about 0.5 g of Mefloquine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.48 mg of $C_{17}H_{16}F_6N_2O.HCl$

Containers and storage Containers—Well-closed containers.

Mefruside

メフルシド



and enantiomer

$C_{13}H_{19}ClN_2O_5S_2$: 382.88

4-Chloro-*N*-methyl-*N*-[(2*RS*)-2-methyltetrahydrofuran-2-ylmethyl]-3-sulfamoylbenzenesulfonamide

[*7195-27-9*]

Mefruside, when dried, contains not less than 98.5% of $C_{13}H_{19}ClN_2O_5S_2$.

Description Mefruside occurs as a white crystalline powder.

It is very soluble in dimethylformamide, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

A solution of Mefruside in dimethylformamide (1 in 10) has no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Mefruside in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mefruside, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Mefruside as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 149 – 152°C

Purity **(1)** Heavy metals <*1.07*>—Dissolve 1.0 g of Mefruside 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, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Mefruside according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone 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 <*2.03*>. Spot 10 μL each of the sample 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 and acetone (5:2) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Mefruside, previously dried, dissolve in 80 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 13 mL of water to 80 mL of *N*,*N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 38.29 mg of $C_{13}H_{19}ClN_2O_5S_2$

Containers and storage Containers—Well-closed containers.

Mefruside Tablets

メフルシド錠

Mefruside Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$: 382.88).

Method of preparation Prepare as directed under Tablets, with Mefruside.

Identification **(1)** Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside according to the labeled amount, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts <*2.60*> between 149°C and 152°C.

(2) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside according to the labeled amount, shake with 70 mL of methanol strongly for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 274 nm and 278 nm, and between 283 nm and 287 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mefruside Tablets add 40 mL of methanol, disintegrate the tablet using ultrasonic waves with occasional stirring, then further treat with ultrasonic waves for 10 minutes, and add methanol to make exactly V mL of a solution containing about 0.5 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) per mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, add methanol to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
$= M_S \times A_T/A_S \times V/125$

M_S: Amount (mg) of mefruside for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mefruside Tablets is not less than 85%.

Start the test with 1 tablet of Mefruside Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a filter paper for quantitative analysis (5C). Discard the first 5 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 mefruside ($C_{13}H_{19}ClN_2O_5S_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 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 sample solution and standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of mefruside for assay
C: Labeled amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) in 1 tablet

Assay Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 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 the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

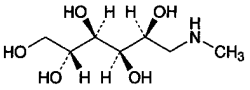
Amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of mefruside for assay

Containers and storage Containers—Tight containers.

Meglumine

メグルミン



$C_7H_{17}NO_5$: 195.21
1-Deoxy-1-methylamino-D-glucitol
[*6284-40-8*]

Meglumine, when dried, contains not less than 99.0% of $C_7H_{17}NO_5$.

Description Meglumine occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, and slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Meglumine (1 in 10) is between 11.0 and 12.0.

Identification **(1)** To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

(2) To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

(3) Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts <2.60> between 149°C and 152°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: −16.0 – −17.0° (after drying, 1 g, water, 10 mL, 100 mm).

Melting point <2.60> 128 – 131°C

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

(2) Chloride <1.03>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 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 0.009%).

(3) Sulfate <1.14>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 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 with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Meglumine 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 <1.11>—Prepare the test solution with 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

(6) Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling's TS, and boil for 2 minutes: no red-brown precipitate is produced.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS = 19.52 mg of $C_7H_{17}NO_5$

Containers and storage Containers—Tight containers.

Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

Method of preparation

(1)	
Iotalamic Acid	227.59 g
Meglumine	72.41 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL
(2)	
Iotalamic Acid	455 g
Meglumine	145 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification **(1)** To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium naphthoquinone sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamic Acid.

Optical rotation ⟨*2.49*⟩
Method of preparation (1) α_D^{20}: −1.67 – −1.93° (100 mm).
Method of preparation (2) α_D^{20}: −3.35 – −3.86° (100 mm).

pH ⟨*2.54*⟩ 6.5 – 7.7

Purity **(1)** Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.
(2) Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, and proceed as directed in the Purity (2) under Sodium Iotalamate Injection.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Meglumine Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of iotalamic acid to that of the internal standard.

$$\text{Amount (mg) of iotalamic acid } (C_{11}H_9I_3N_2O_4) = M_S \times Q_T/Q_S \times 10$$

M_S: Amount (mg) of iotalamic acid for assay

Internal standard solution—A solution of L-tryptophan in the mobile phase (3 in 2500).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside 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 20°C.
Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of iotalamic acid 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, iotalamic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Meglumine Sodium Amidotrizoate Injection

アミドトリゾ酸ナトリウムメグルミン注射液

Meglumine Sodium Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

Method of preparation

(1)	
Amidotrizoic Acid (anhydrous)	471.78 g
Sodium Hydroxide	5.03 g
Meglumine	125.46 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL
(2)	
Amidotorizoic Acid (anhydrous)	597.30 g
Sodium Hydroxide	6.29 g
Meglumine	159.24 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification **(1)** To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(3) Meglumine Sodium Amidotrizoate Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*>

Method of preparation (1) α_D^{20}: -2.91 – $-3.36°$ (100 mm).

Method of preparation (2) α_D^{20}: -3.69 – $-4.27°$ (100 mm).

pH <*2.54*> 6.0 – 7.7

Purity **(1)** Primary aromatic amines—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.20 g of Amidotrizoic Acid according to the labeled amount, add 6 mL of water, mix, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

(2) Iodine and iodide—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.25 g of Amidotrizoic Acid according to the labeled amount, add water to make 20 mL, add 5 mL of dilute nitric acid, shake well, and filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Meglumine Sodium Amidotrizoate Injection, equivalent to about 0.5 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 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 sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying <*2.41*> in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) 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 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amidotrizoic acid to that of the internal standard.

$$\text{Amount (mg) of amidotrizoic acid } (C_{11}H_9I_3N_2O_4) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

Internal standard solution—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid 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, amidotrizoic acid 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Meglumine Sodium Iodamide Injection

ヨーダミドナトリウムメグルミン注射液

Meglumine Sodium Iodamide Injection is an aqueous solution for injection.

It contains not less than 59.7 w/v% and not more than 65.9 w/v% of iodamide ($C_{12}H_{11}I_3N_2O_4$: 627.94).

Method of preparation

Iodamide	627.9 g
Sodium Hydroxide	6.0 g
Meglumine	165.9 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Meglumine Sodium Iodamide Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification **(1)** To 2 mL of Meglumine Sodium Iodamide Injection add 25 mL of water, and add 3 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is formed. Filter the precipitate by suction through a glass filter (G3), and wash with two 10-mL portions of water. Transfer the precipitate to a suitable flask, add 100 mL of water, dissolve by heating, and gently boil until the volume becomes about 30 mL. After cooling, collect the separated crystals by filtration, dry at 105°C for 1 hour, and proceed as directed in the Identification (1) and (2) under Iodamide.

(2) Determine the infrared absorption spectrum of the dried crystals obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 3390 cm^{-1}, 1369 cm^{-1}, 1296 cm^{-1}, 1210 cm^{-1} and 1194 cm^{-1}.

(3) To 1 mL of Meglumine Sodium Iodamide Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color is produced.

(4) Meglumine Sodium Iodamide Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> α_D^{20}: −3.84 – −4.42° (100 mm).

pH <*2.54*> 6.5 – 7.5

Purity **(1)** Primary aromatic amines—Mix 0.30 mL of Meglumine Sodium Iodamide Injection and 6 mL of water, then add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and proceed as directed in the Purity (2) under Iodamide: the absorbance is not more than 0.22.

(2) Iodine and iodide—To 0.40 mL of Meglumine Sodium Iodamide Injection add water to make 20 mL, then add 5 mL of dilute nitric acid, shake well, filter by suction through a glass filter (G3). To the filtrate add 5 mL of chloroform, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of a strong hydrogen peroxide solution, and shake vigorously: the chloroform layer has no more color than the control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. To a 0.10-mL portion of this solution add 20 mL of water, 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of strong hydrogen peroxide solution, and shake vigorously.

Extractable volume <*6.05*> It meets the requirement.

Pyrogen <*4.04*> Dilute Meglumine Sodium Iodamide Injection with isotonic sodium chloride solution so as to contain 0.30 mL of Meglumine Sodium Iodamide Injection per mL according to the labeled amount, and perform the test: it meets the requirements.

Assay To an exactly measured 8 mL of Meglumine Sodium Iodamide Injection add sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution into a saponification flask, add 30 mL of sodium hydroxide TS and 1 g of zinc powder, and proceed as directed in the Assay under Iodamide.

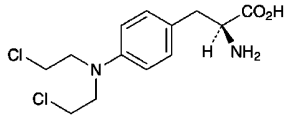
Each mL of 0.1 mol/L silver nitrate VS
= 20.93 mg of $C_{12}H_{11}I_3N_2O_4$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Melphalan

メルファラン



$C_{13}H_{18}Cl_2N_2O_2$: 305.20
4-Bis(2-chloroethyl)amino-L-phenylalanine
[*148-82-3*]

Melphalan contains not less than 93.0% of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried basis.

Description Melphalan occurs as a white, to light yellowish white, crystalline powder.

It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS.

It is gradually colored by light.

Optical rotation $[\alpha]_D^{20}$: about −32° (0.5 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification **(1)** To 20 mg of Melphalan add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for chloride.

(3) Determine the absorption spectrum of a solution of Melphalan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and conpare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of diluted nitric acid

(1 in 40), stir for 2 minutes, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Melphalan 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 <1.11>—Prepare the test solution with 1.0 g of Melphalan according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

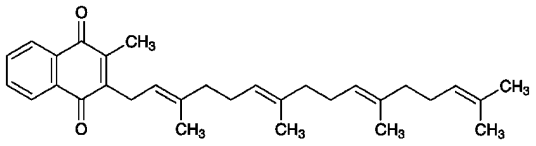
Assay Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS
= 15.26 mg of $C_{13}H_{18}Cl_2N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Menatetrenone

メナテトレノン



$C_{31}H_{40}O_2$: 444.65
2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone
[*863-61-6*]

Menatetrenone contains not less than 98.0% of $C_{31}H_{40}O_2$, calculated on the dehydrated basis.

Description Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about 37°C.

Identification (1) Dissolve 0.1 g of Menatetrenone in 5 mL of ethanol (99.5) by warming, cool, and add 1 mL of a solution of potassium hydroxide in ethanol (95) (1 in 10): a blue color develops, and upon standing it changes from blue-purple to red-brown through red-purple.

(2) Determine the infrared absorption spectrum of Menatetrenone, after melting by warming if necessary, as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Menatetrenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Menatetrenone 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) Menadione—To 0.20 g of Menatetrenone add 5 mL of diluted ethanol (1 in 2), shake well, and filter. To 0.5 mL of the filtrate add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazorone in ethanol (99.5) (1 in 20) and 1 drop of ammonia water (28), and allow to stand for 2 hours: no blue-purple color develops.

(3) *cis* Isomer—Dissolve 0.10 g of Menatetrenone in 10 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane 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 <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of hexane and dibutyl ether (17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot corresponding to relative *R*f value 1.1 regarding to the principal spot from the sample solution is not more intense than the spot from the standard solution.

(4) Related substances—Conduct this procedure without exposure to daylight, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 menatetrenone from the sample solution is not larger than the peak area of menatetrenone 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.

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

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

Water <2.48> Not more than 0.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of menatetrenone to that of the internal standard.

$$\text{Amount (mg) of } C_{31}H_{40}O_2 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Menatetrenone RS, calculated on the dehydrated basis

Internal standard solution—A solution of phytonadione in 2-propanol (1 in 20,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Methanol.

Flow rate: Adjust the flow rate so that the retention time of menatetrenone 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, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

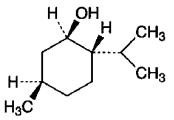
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

dl-Menthol

dl-メントール



and enantiomer

$C_{10}H_{20}O$: 156.27

(1*RS*,2*SR*,5*RS*)-5-Methyl-2-(1-methylethyl)cyclohexanol

[*89-78-1*]

dl-Menthol contains not less than 98.0% of $C_{10}H_{20}O$.

Description *dl*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification **(1)** Triturate *dl*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of *dl*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

Congealing point ⟨*2.42*⟩ 27 – 28°C

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −2.0 – +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Purity **(1)** Non-volatile residue—Volatilize 2.0 g of *dl*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of *dl*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of *dl*-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N*,*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

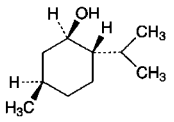
Assay Weigh accurately about 2 g of *dl*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of $C_{10}H_{20}O$

Containers and storage Containers—Tight containers.
Storage—In a cold place.

l-Menthol

l-メントール



$C_{10}H_{20}O$: 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol
[*2216-51-5*]

l-Menthol contains not less than 98.0% of $C_{10}H_{20}O$.

Description *l*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification **(1)** Triturate *l*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of *l*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −45.0 – −51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Melting point <*2.60*> 42 – 44°C

Purity **(1)** Non-volatile residue—Volatilize 2.0 g of *l*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of *l*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of *l*-Menthol placed in a flask add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N*,*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

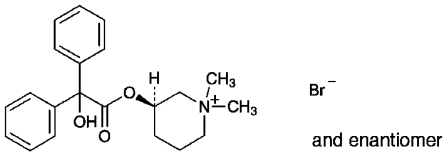
Assay Weigh accurately about 2 g of *l*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash the condenser with 20 mL of water, and titrate <*2.50*> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of $C_{10}H_{20}O$

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Mepenzolate Bromide

メペンゾラート臭化物



$C_{21}H_{26}BrNO_3$: 420.34
(3*RS*)-3-[(Hydroxy)(diphenyl)acetoxy]-1,1-dimethylpiperidinium bromide
[*76-90-4*]

Mepenzolate Bromide, when dried, contains not less than 98.5% of $C_{21}H_{26}BrNO_3$.

Description Mepenzolate Bromide is white to pale yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in hot water, slightly soluble in water and in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 230°C (with decomposition).

Identification **(1)** To 30 mg of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

(2) Dissolve 10 mg of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff's TS: an orange precipitate is produced.

(3) Determine the absorption spectrum of a solution of Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests <*1.09*> for Bromide.

Purity **(1)** Heavy Metals <*1.07*>—Proceed with 1.0 g of Mepenzolate Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not less than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Mepenzolate Bromide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use

this solution as the standard solution (1). Separately, dissolve 40 mg of benzophenone in 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 (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorecent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100) (3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

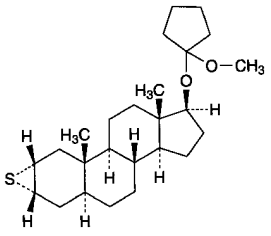
Assay Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.03 mg of $C_{21}H_{26}BrNO_3$

Containers and storage Containers—Tight containers.

Mepitiostane

メピチオスタン



$C_{25}H_{40}O_2S$: 404.65
2α,3α-Epithio-17β-(1-methoxycyclopentyloxy)-5α-androstane
[*21362-69-6*]

Mepitiostane contains not less than 96.0% and not more than 102.0% of $C_{25}H_{40}O_2S$, calculated on the anhydrous basis.

Description Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

Identification **(1)** Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2) Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt <*2.60*> between 144°C and 149°C.

(3) Determine the infrared absorption spectrum of Mepitiostane as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Mepitiostane 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 20 mg of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitiostanol RS in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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 hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same *R*f value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

Water <*2.48*> Not more than 0.7% (0.3 g, volumetric titration, back titration).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL

of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol RS, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of epitiostanol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{25}H_{40}O_2S = M_S \times Q_T/Q_S \times 5 \times 1.320$$

M_S: Amount (mg) of Epitiostanol RS, calculated on the anhydrous basis

Internal standard solution—A solution of *n*-octylbenzene in ethanol (99.5) (1 in 300).

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside diameter and 15 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 methanol and water (20:3).

Flow rate: Adjust the flow rate so that the retention time of epitiostanol 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, epitiostanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

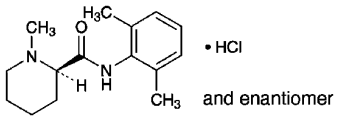
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epitiostanol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Mepivacaine Hydrochloride

メピバカイン塩酸塩



$C_{15}H_{22}N_2O.HCl$: 282.81

(2*RS*)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide monohydrochloride

[*1722-62-9*]

Mepivacaine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{22}N_2O.HCl$.

Description Mepivacaine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 256°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Mepivacaine Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mepivacaine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.2 g of Mepivacaine Hydrochloride 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 Mepivacaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Mepivacaine 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).

(4) Related substances—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol 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 <2.03>. Spot 10 μL each of the sample solution and standard

solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol and ammonia solution (28) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 mL of acetic anhydride. Titrate ⟨*2.50*⟩ 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
= 28.28 mg of $C_{15}H_{22}N_2O.HCl$

Containers and storage Containers—Tight containers.

Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Mepivacaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$: 282.81).

Method of preparation Prepare as directed under Injections, with Mepivacaine Hydrochloride.

Description Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Mepivacaine Hydrochloride Injection, equivalent to 20 mg of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydrochloride TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

pH Being specified separately.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.6 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$), add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloride TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of mepivacaine to that of the internal standard.

Amount (mg) of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of mepivacaine hydrochloride for assay

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 15 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 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

System suitability—

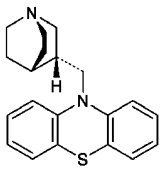
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, mepivacaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of mepivacaine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Mequitazine

メキタジン



and enantiomer

$C_{20}H_{22}N_2S$: 322.47
10-[(3*RS*)-1-Azabicyclo[2.2.2]oct-3-ylmethyl]-10*H*-phenothiazine
[*29216-28-2*]

Mequitazine, when dried, contains not less than 98.5% of $C_{20}H_{22}N_2S$.

Description Mequitazine occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.

It is gradually colored by light.

A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mequitazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 146 – 150°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mequitazine 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—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Mequitazine in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, then pipet 5 mL of this solution, add methanol 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

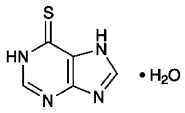
Assay Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.25 mg of $C_{20}H_{22}N_2S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Mercaptopurine Hydrate

メルカプトプリン水和物



$C_5H_4N_4S.H_2O$: 170.19
1,7-Dihydro-6*H*-purine-6-thione monohydrate
[*6112-76-1*]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine ($C_5H_4N_4S$: 152.18), calculated on the anhydrous basis.

Description Mercaptopurine Hydrate occurs as light yellow to yellow crystals or crystalline powder. It is odorless.

It is practically insoluble in water, in acetone and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification **(1)** Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <*2.60*> between 218°C and 222°C (with decomposition).

(2) Determine the absorption spectrum of a solution of Mercaptopurine Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) Sulfate <*1.14*>—Dissolve 50 mg of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Mercaptopurine 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) Hypoxanthine—Dissolve 50 mg of Mercaptopurine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, *n*-butyl formate and ammonia solution (28) (8:6:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution observed at the same place as that from the standard solution, is not larger and not more intense than that from the standard solution.

(5) Phosphorus—Take 0.20 g of Mercaptopurine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogenphosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexaammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank: the absorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

Water <*2.48*> 10.0 – 12.0% (0.2 g, volumetric titration, back titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of *N*,*N*-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 15.22 mg of $C_5H_4N_4S$

Containers and storage Containers—Well-closed containers.

Mercurochrome

Merbromin

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

Description Mercurochrome occurs as blue-green to greenish red-brown scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

Identification **(1)** A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

(2) To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

(3) Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

(4) Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chlorine TS and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

Purity **(1)** Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

(2) Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

(3) Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

(4) Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24 hours, centrifuge, and wash the precipitate with small por-

tions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 5 hours).

Assay (1) Mercury—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to an iodine flask, dissolve in 50 mL of water, add 8 mL of acetic acid (31), 20 mL of chloroform and exactly 30 mL of 0.05 mol/L iodine VS, stopper tightly, and allow to stand for 1 hour with frequent, vigorous shaking. Titrate ⟨*2.50*⟩ the excess iodine with 0.1 mol/L sodium thiosulfate VS with vigorous shaking (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

(2) Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate ⟨*2.50*⟩ 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 = 7.990 mg of Br

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Mercurochrome Solution

Merbromin Solution

マーキュロクロム液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

Method of preparation

Mercurochrome	20 g
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare by mixing the above ingredients.

Description Mercurochrome Solution is a dark red liquid.

Identification (1) To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

(2) Dilute 1 mL of Mercurochrome Solution with 4 mL of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

(3) Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

(4) To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

Purity Dyestuff—To 20 mL of Mercurochrome Solution add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

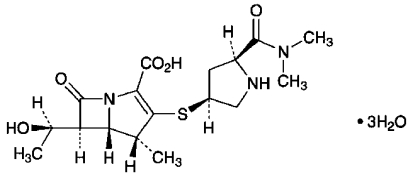
Assay Transfer exactly measured 30 mL of Mercurochrome Solution to an iodine flask, dilute with 20 mL of water, add 8 mL of acetic acid (31) and 20 mL of chloroform, and proceed as directed in the Assay (1) under Mercurochrome.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Meropenem Hydrate

メロペネム水和物



$C_{17}H_{25}N_3O_5S.3H_2O$: 437.51

(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-ylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate [*119478-56-7*]

Meropenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Meropenem Hydrate is expressed as mass (potency) of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

Description Meropenem Hydrate occurs as a white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 10 mg of Meropenem Hydrate in 2 mL of water, add 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) Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem RS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Meropenem Hydrate and Meropenem RS as directed in the potassium bromide disk method under Infrared Spectropho-

tometry ⟨*2.25*⟩, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -17 – $-21°$ (0.22 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.2 g of Meropenem Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Meropenem Hydrate in 10 mL of sodium hydrogen carbonate TS: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Meropenem 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) Related substances—Dissolve 50 mg of Meropenem Hydrate in 10 mL of triethylamine-phosphate buffer solution, pH 5.0, and use this solution as the sample solution. Prepare the sample solution before use. Pipet 1 mL of the sample solution, and add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 100 mL. Pipet 3 mL of this solution, add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of ring-opened meropenem, having the relative retention time about 0.5 to meropenem, and the peak area of the dimmer, having the relative retention time about 2.2 to meropenem, obtained from the sample solution are not larger than the peak area of meropenem from the standard solution, the area of the peak other than meropenem and the peaks mentioned above from the sample solution is not larger than 1/3 times the peak area of meropenem from the standard solution, and the total area of the peaks other than meropenem from the sample solution is not larger than 3 times the peak area of meropenem from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside 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 triethylamine-phosphate buffer solution, pH 5.0 and acetonitrile (100:7).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 6 minutes.

Time span of measurement: About 7 times as long as the retention time of meropenem.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 25 mL. Confirm that the peak area of meropenem obtained from 10 μL of this solution is equivalent to 16 to 24% of that from 10 μL of the standard solution.

System performance: Warm the sample solution at 60°C for 30 minutes. When the procedure is run with 10 μL of the sample solution under the above operating conditions, the ring-opened meropenem, meropenem and the dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is not less than 1.5.

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

Water ⟨*2.48*⟩ Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Meropenem Hydrate and Meropenem RS, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add triethylamine-phosphate buffer solution, pH 5.0 to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of meropenem to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of meropenem } (C_{17}H_{25}N_3O_5S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Meropenem RS

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside 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 triethylamine-phosphate buffer solution, pH 5.0 and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of meropenem 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, meropenem 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 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Meropenem for Injection

注射用メロペネム

Meropenem for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

Method of preparation Prepare as directed under Injections, with Meropenem Hydrate.

Description Meropenem for Injection occurs as a white to light yellow crystalline powder.

Identification Determine the infrared absorption spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3410 cm^{-1}, 1750 cm^{-1}, 1655 cm^{-1}, 1583 cm^{-1} and 1391 cm^{-1}.

pH ⟨*2.54*⟩ Dissolve an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of Meropenem Hydrate according to the labeled amount, in 5 mL of water: the pH of the solution is between 7.3 and 8.3.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate according to the labeled amount, in 20 mL of water: the solution is clear and is not more intensely colored than the following matching fluid.

Matching fluid: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2) Related substances—Being specified separately.

Loss on drying ⟨*2.41*⟩ 9.5 – 12.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.12 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass on the contents of not less than 10 containers of Meropenem for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Meropenem Hydrate, dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Meropenem RS, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of meropenem to that of the internal standard.

Amount [mg (potency)] of meropenem ($C_{17}H_{25}N_3O_5S$)
$= M_S \times Q_T/Q_S$

M_S: Amount [mg (potency)] of Meropenem RS

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Meropenem Hydrate.

System suitability—

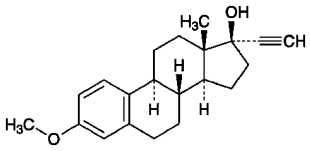
System performance: Proceed as directed in the system suitability in the Assay under Meropenem Hydrate.

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 ratio of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Mestranol

メストラノール



$C_{21}H_{26}O_2$: 310.43

3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol

[*72-33-3*]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of $C_{21}H_{26}O_2$.

Description Mestranol occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

(2) Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mestranol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mestranol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or

the spectrum of previously dried Mestranol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +2 – +8° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 148 – 154°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Mestranol 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Mestranol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Mestranol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg each of Mestranol and Mestranol RS, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

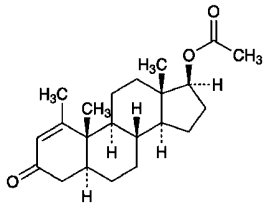
$$\text{Amount (mg) of } C_{21}H_{26}O_2 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Mestranol RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Acetate

メテノロン酢酸エステル



$C_{22}H_{32}O_3$: 344.49
1-Methyl-3-oxo-5α-androst-1-en-17β-yl acetate
[*434-05-9*]

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

Description Metenolone Acetate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

Identification **(1)** Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

(2) To 10 mg of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Dissolve 50 mg of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts ⟨*2.60*⟩ between 157°C and 161°C.

(4) Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +39 – +42° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 141 – 144°C

Purity **(1)** Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Metenolone Acetate 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 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the

sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample 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 and cyclohexane (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

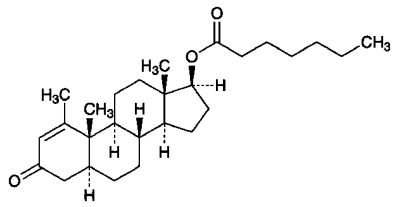
Assay Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of $C_{22}H_{32}O_3 = A/391 \times 10{,}000$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Enanthate

メテノロンエナント酸エステル



$C_{27}H_{42}O_3$: 414.62
1-Methyl-3-oxo-5α-androst-1-en-17β-yl heptanoate
[*303-42-4*]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{27}H_{42}O_3$.

Description Metenolone Enanthate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

Identification (1) Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1) on a water bath for 30 minutes: a red-brown color develops.

(2) Dissolve 0.05 g of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts <2.60> between 156°C and 162°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: +39 – +43° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 67 – 72°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone Enanthate 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 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1:1) 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 does not appear.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance, A, of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of $C_{27}H_{42}O_3 = A/325 \times 100{,}000$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Enanthate Injection

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of metenolone enanthate ($C_{27}H_{42}O_3$: 414.62).

Method of preparation Prepare as directed under Injections, with Metenolone Enanthate.

Description Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

Identification (1) Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate according to the labeled amount, add 20 mL of

petroleum ether, and extract with three 20-mL portions of diluted acetic acid (100) (5 in 7). Combine the extracts, wash with 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

(2) Measure a volume of Metenolone Enanthate Injection, equivalent to 10 mg of Metenolone Enanthate according to the labeled amount, dissolve in 10 mL of chloroform, and use this solution as the sample solution. Separately dissolve 10 mg of metenolone enanthate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 15 cm, and air-dry the plate. Again develop this plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same *R*f value.

Extractable volume <*6.05*> It meets the requirement.

Assay To an exactly measured volume of Metenolone Enanthate Injection, equivalent to about 0.1 g of metenolone enanthate ($C_{27}H_{42}O_3$), add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 0.1 g of metenolone enanthate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 3 mL each of the sample solution and standard solution, and treat each solution as follows: add 10 mL of isoniazid TS, exactly measured, add methanol to make exactly 20 mL, and allow to stand for 60 minutes. Determine the absorbances, A_T and A_S, of the solutions from the sample solution and standard solution, respectively, at 384 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution obtained by proceeding with 3 mL of chloroform as the blank.

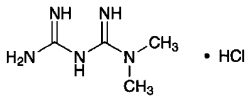
$$\text{Amount (mg) of metenolone enanthate } (C_{27}H_{42}O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of metenolone enanthate for assay

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Metformin Hydrochloride

メトホルミン塩酸塩



$C_4H_{11}N_5.HCl$: 165.62
1,1-Dimethylbiguanide monohydrochloride
[*1115-70-4*]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_4H_{11}N_5.HCl$.

Description Metformin Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Metformin Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Metformin 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).

(2) Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution (1), the num-

ber of them showing more intense than the spot with the standard solution (2) is not more than two, and the spot with the sample solution appeared at the position corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Metformin Hydrochloride, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 4.141 mg of $C_4H_{11}N_5$.HCl

Containers and storage Containers—Tight containers.

Metformin Hydrochloride Tablets

メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5$.HCl: 165.62).

Method of preparation Prepare as directed under Tablets, with Metformin Hydrochloride.

Identification Shake an amount of powdered Metformin Hydrochloride Tablets, equivalent to 250 mg of Metformin Hydrochloride according to the labeled amount, with 25 mL of 2-propanol, and filter. Evaporate the filtrate under reduced pressure in a water bath at 40°C, and determine the infrared absorption spectrum of the residue as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3370 cm^{-1}, 3160 cm^{-1}, 1627 cm^{-1}, 1569 cm^{-1} and 1419 cm^{-1}.

Uniformity of dosage unit ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of metformin hydrochloride ($C_4H_{11}N_5$.HCl), add 70 mL of a mixture of water and acetonitrile (3:2), shake for 10 minutes, add the mixture of water and acetonitrile (3:2) to make exactly 100 mL, 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 3 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the mixture of water and acetonitrile (3:2) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of metformin to that of the internal standard.

Amount (mg) of metformin hydrochloride ($C_4H_{11}N_5$.HCl)
= $M_S \times Q_T/Q_S$

M_S: Amount (mg) of metformin hydrochloride for assay

Internal standard solution—Dissolve 0.3 g of isobutyl parahydroxybenzoate in 100 mL of the mixture of water and acetonitrile (3:2).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 0.8 g of sodium lauryl sulfate in 620 mL of diluted phosphoric acid (1 in 2500), and add 380 mL of acetonitrile.

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

System suitability—

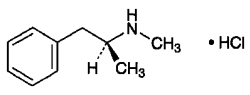
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, metformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Methamphetamine Hydrochloride

メタンフェタミン塩酸塩



$C_{10}H_{15}N$.HCl: 185.69
(2*S*)-*N*-Methyl-1-phenylpropan-2-amine monohydrochloride
[*51-57-0*]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5% of $C_{10}H_{15}N$.HCl.

Description Methamphetamine Hydrochloride occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in chlo-

roform, and practically insoluble in diethyl ether.

The pH of a solution of Methamphetamine Hydrochloride (1 in 10) is between 5.0 and 6.0.

Identification **(1)** To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of hydrogen hexachloroplatinate (IV) TS: an orange-yellow, crystalline precipitate is produced.

(2) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of iodine TS: a brown precipitate is produced.

(3) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of 2,4,6-trinitrophenol TS: a yellow, crystalline precipitate is produced.

(4) A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +16 – +19° (after drying, 0.2 g, water, 10 mL, 100 mm).

Melting point <*2.60*> 171 – 175°C

Purity **(1)** Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(i) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

(2) Sulfate <*1.14*>—Dissolve 0.05 g of Methamphetamine 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: the solution remains unchanged.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

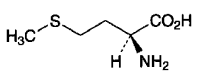
Assay Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.57 mg of $C_{10}H_{15}N.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

L-Methionine

L-メチオニン



$C_5H_{11}NO_2S$: 149.21
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid
[*63-68-3*]

L-Methionine, when dried, contains not less than 98.5% of $C_5H_{11}NO_2S$.

Description L-Methionine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Methionine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +21.0 – +25.0° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 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, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Methionine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Methionine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by warming, cool, and add 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 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <*1.11*>—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, immediately 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 evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

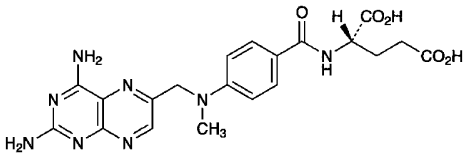
Assay Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.92 mg of $C_5H_{11}NO_2S$

Containers and storage Containers—Tight containers.

Methotrexate

メトトレキサート



$C_{20}H_{22}N_8O_5$: 454.44
N-{4-[(2,4-Diaminopteridin-6-ylmethyl)(methyl)amino]benzoyl}-L-glutamic acid
[*59-05-2*]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

Description Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

Identification **(1)** Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water ⟨*2.48*⟩ Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 25 mg each of Methotrexate and Methotrexate RS, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of methotrexate in each solution.

Amount (mg) of $C_{20}H_{22}N_8O_5 = M_S \times A_T/A_S$

M_S: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside 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 disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, folic acid and methotrexate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methotrexate Capsules

メトトレキサートカプセル

Methotrexate Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$: 454.44).

Method of preparation Prepare as directed under Capsules, with Methotrexate.

Identification To an amount of the content of Methotrexate Capsules, equivalent to 2 mg of Methotrexate according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 10 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 240 nm and 244 nm and between 304 nm and 308 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To the content of 1 capsule of Methotrexate Capsules add $3V/5$ mL of the mobile phase, agitate with the aid of ultrasonic waves for 15 minutes, then shake for 25 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 20 μg of methotrexate ($C_{20}H_{22}N_8O_5$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <*2.48*> in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methotrexate to that of the internal standard.

$$\text{Amount (mg) of methotrexate } (C_{20}H_{22}N_8O_5) = M_S \times Q_T/Q_S \times V/500$$

M_S: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

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

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methotrexate to that of the internal standard is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methotrexate Capsules is not less than 85%.

Start the test with 1 capsule of Methotrexate Capsules, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.2 μg of methotrexate ($C_{20}H_{22}N_8O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <*2.48*> in the same manner as Methotrexate), and dissolve in the mobile phase 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of methotrexate of both solutions.

$$\text{Dissolution rate (\%) with respect to the labeled amount of methotrexate } (C_{20}H_{22}N_8O_5) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$) in 1 capsule

Operating conditions—

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 the symmetry factor of the peak of methotrexate are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Methotrexate Capsules, take out all of the content, and accurately weigh the mass of the empty capsules. Powder the content, weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate ($C_{20}H_{22}N_8O_5$), add 60 mL of the mobile phase, shake for 25 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <*2.48*> in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, 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 <*2.01*>, and calculate the ratios, Q_T and Q_S, of the peak area of methotrexate to that of the internal standard.

$$\text{Amount (mg) of methotrexate } (C_{20}H_{22}N_8O_5) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Internal standard solution—A solution of 4-nitrophenol in

methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside 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: To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 28.5 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 6 minutes.

System suitability—

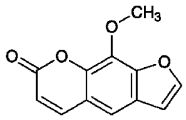
System performance: Dissolve 10 mg each of methotrexate and folic acid in 100 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methotrexate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Methoxsalen

メトキサレン



$C_{12}H_8O_4$: 216.19

9-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one

[*298-81-7*]

Methoxsalen contains not less than 98.0% and not more than 102.0% of $C_{12}H_8O_4$, calculated on the anhydrous basis.

Description Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification **(1)** To 10 mg of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2) To 10 mg of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3) Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 145 – 149°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Methoxsalen 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 <*1.11*>—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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, hexane and ethyl acetate (40:10:3) 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 sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen RS, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{12}H_8O_4 = M_S \times A_T/A_S$$

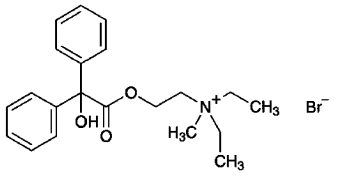
M_S: Amount (mg) of Methoxsalen RS, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylbenactyzium Bromide

メチルベナクチジウム臭化物



$C_{21}H_{28}BrNO_3$: 422.36
N,*N*-Diethyl-2-[(hydroxyl)(diphenyl)acetoxy]-*N*-methylethylaminium bromide
[*3166-62-9*]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of $C_{21}H_{28}BrNO_3$.

Description Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 50) is between 5.0 and 6.0.

Identification **(1)** Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2) To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt ⟨*2.60*⟩ between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3) Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for bromide.

Melting point ⟨*2.60*⟩ 168 – 172°C

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

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Methylbenactyzium Bromide 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).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (2 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate ⟨*2.50*⟩ 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
= 42.24 mg of $C_{21}H_{28}BrNO_3$

Containers and storage Containers—Tight containers.

Methylcellulose

Cellulose, methyl ether

メチルセルロース

[*9004-67-5*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Methylcellulose is a methyl ether of cellulose.

It contains not less than 26.0% and not more than 33.0% of methoxy group (-OCH_3: 31.03), calculated on the dried basis.

The viscosity of Methylcellulose is shown in millipascal second (mPa·s).

◆**Description** Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.◆

Identification **(1)** Disperse evenly 1.0 g of Methylcellulose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of diluted sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water.Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color, and it does not change to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

Viscosity ⟨*2.53*⟩

(i) Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-

revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model.

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)	Rotor No.	Retation frequency /min	Conversion factor
Not less than 600 and less than 1400	3	60	20
〃 1400 〃 3500	3	12	100
〃 3500 〃 9500	4	60	100
〃 9500 〃 99,500	4	6	1000
〃 99,500	4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

pH ⟨*2.54*⟩ Allow the sample solution obtained in the Viscosity to stand at 20 ± 2°C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

Purity Heavy metals—Put 1.0 g of Methylcellulose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid in a reaction bottle, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of iodomethane to that of the internal standard.

$$\text{Content (\%) of methoxy group } (CH_3O) = M_S/M \times Q_T/Q_S \times 21.86$$

M_S: Amount (mg) of iodomethane in the standard solution

M: Amount (mg) of sample, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

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

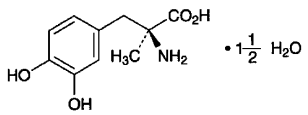
System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane and the internal standard are eluted in this order, with complete separation of these peaks.

◆**Containers and storage** Containers—Well-closed containers.◆

Methyldopa Hydrate

メチルドパ水和物



$C_{10}H_{13}NO_4.1\frac{1}{2}H_2O$: 238.24

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate

[*41372-08-1*]

Methyldopa Hydrate contains not less than 98.0% of methyldopa ($C_{10}H_{13}NO_4$: 211.21), calculated on the anhydrous basis.

Description Methyldopa Hydrate occurs as a white to pale grayish white, crystalline powder.

It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification **(1)** To 10 mg of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

(2) Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.44*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Methyldopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyldopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −25 – −28° (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

Purity **(1)** Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Methyldopa 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 <*1.11*>—Prepare the test solution with 1.0 g of Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).

(5) 3-*O*-Methylmethyldopa—Dissolve 0.10 g of Methyldopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa for thin-layer chromatography 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 <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

Water <*2.48*> 10.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 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
= 21.12 mg of $C_{10}H_{13}NO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methyldopa Tablets

メチルドパ錠

Methyldopa Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$: 211.21).

Method of preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification **(1)** To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate according to the labeled amount, add 10 mL of water, and heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

(2) To 0.5 mL of the supernatant liquid obtained in the Identification (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

(3) To 0.7 mL of the supernatant liquid obtained in the Identification (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 283 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyldopa Tablets add 50 mL of 0.05 mol/L sulfuric acid TS, shake for 15 minutes, then add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 5 mg of methyldopa ($C_{10}H_{13}NO_4$), add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (separately determine the loss on drying <*2.41*> at 125°C for 2 hours), and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 520 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$)
$= M_S \times A_T/A_S \times 5/V$

M_S: Amount (mg) of Methyldopa RS, calculated on the dried basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Methyldopa Tablets is not less than 75%.

Start the test with 1 tablet of Methyldopa Tablets, withdraw not less than 30 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 25 μg of methyldopa ($C_{10}H_{13}NO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (separately determine the loss on drying <*2.41*> at 125°C for 2 hours), and dissolve in water to make exactly 200 mL. 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 sample solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of methyldopa ($C_{10}H_{13}NO_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of methyldopa for assay, calculated on the dried basis

C: Labeled amount (mg) of methyldopa ($C_{10}H_{13}NO_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa ($C_{10}H_{13}NO_4$), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dry at 125°C for 2 hours, and determine the loss on drying <*2.41*>), dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

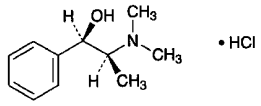
Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$)
$= M_S \times A_T/A_S$

M_S: amount (mg) of Methyldopa RS, calculated on the dried basis

Containers and storage Containers—Well-closed containers.

dl-Methylephedrine Hydrochloride

dl-メチルエフェドリン塩酸塩



and enantiomer

$C_{11}H_{17}NO.HCl$: 215.72
(1*RS*,2*SR*)-2-Dimethylamino-1-phenylpropan-1-ol monohydrochloride
[*18760-80-0*]

dl-Methylephedrine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{11}H_{17}NO.HCl$.

Description *dl*-Methylephedrine Hydrochloride occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride.

A solution of *dl*-Methylephedrine Hydrochloride (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of *dl*-Methylephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of *dl*-Methylephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of *dl*-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of *dl*-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Melting point <*2.60*> 207 – 211°C

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of *dl*-Methylephedrine Hydrochloride 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—Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not larger than the peak area of methylephedrine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

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

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of *dl*-Methylephedrine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 21.57 mg of $C_{11}H_{17}NO.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

10% *dl*-Methylephedrine Hydrochloride Powder

dl-Methylephedrine Hydrochloride Powder

dl-メチルエフェドリン塩酸塩散 10%

10% *dl*-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$: 215.72).

Method of preparation

dl-Methylephedrine Hydrochloride	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% *dl*-Methylephedrine Hydrochloride Powder (1 in 200) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 nm.

Assay Weigh accurately about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, if necessary, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios of the peak area, Q_T and Q_S, of methylephedrine to that of the internal standard.

Amount (mg) of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of *dl*-methylephedrine hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine 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, methylephedrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

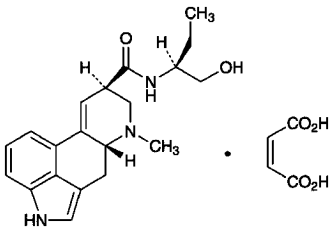
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylergometrine Maleate

メチルエルゴメトリンマレイン酸塩



$C_{20}H_{25}N_3O_2.C_4H_4O_4$: 455.50

(8*S*)-*N*-[(1*S*)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate

[*7054-07-1*]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of $C_{20}H_{25}N_3O_2.C_4H_4O_4$.

Description Methylergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes to yellow by light.

Melting point: about 190°C (with decomposition).

Identification **(1)** A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

(2) The colored solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 5 mL of a solution of Methylergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +44 – +50° (after drying,

0.1 g, water, 20 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 10 mg each of Methylergometrine Maleate and Methylergometrine Maleate RS, previously dried, add water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Pipet 2 mL each of the sample solution and the standard solution separately into brown glassstoppered test tubes, add exactly 4 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while ice cooling, warm for 10 minutes at 45°C, and allow to stand for 20 minutes at room temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 2.0 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

$$\text{Amount (mg) of } C_{20}H_{25}N_3O_2.C_4H_4O_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Methylergometrine Maleate RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Methylergometrine Maleate Tablets

メチルエルゴメトリンマレイン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$: 455.50).

Method of preparation Prepare as directed under Tablets, with Methylergometrine maleate.

Identification **(1)** The sample solution obtained in the Assay shows a blue fluorescence.

(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

Uniformity of dosage unit <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously, and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly V mL of a solution containing about 5 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

$$\text{Amount (mg) of methylergometrine maleate } (C_{20}H_{25}N_3O_2.C_4H_4O_4) = M_S \times A_T/A_S \times V/250$$

M_S: Amount (mg) of Methylergometrine Maleate RS

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methylergometrine Maleate Tablets is not less than 70%.

Start the test with 1 tablet of Methylergometrine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, to exactly V mL of the subsequent filtrate add water to make exactly V' mL so that each mL contains about 0.13 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate RS, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, then pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine immediately the intensities of the

fluorescence, F_T and F_S, of the sample solution and standard solution at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$= M_S \times F_T/F_S \times V'/V \times 1/C \times 9/20$

M_S: Amount (mg) of Methylergometrine Maleate RS
C: Labeled amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$), transfer to a brown separator, add 15 mL of sodium hydrogen carbonate solution (1 in 20), and extract with four 20-mL portions of chloroform. Filter each portion of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform, into another dried, brown separator, combine all the extracts, and use this extract as the sample solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water, and add water to make exactly 100 mL. Pipet 3 mL of this solution, and transfer to a brown separator, proceed in the same manner as the preparation of the sample solution, and use this extract as the standard solution. To each total volume of the sample solution and the standard solution add exactly 25 mL each of dilute *p*-dimethylaminobenzadehyde-ferric chloride TS, and after vigorous shaking for 5 minutes, allow to stand for 30 minutes. Draw off the water layer, centrifuge, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute 4-dimethylaminobenzaldehyde-ferric chloride TS as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

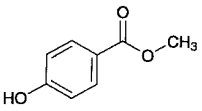
Amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$= M_S \times A_T/A_S \times 3/100$

M_S: Amount (mg) of Methylergometrine Maleate RS

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル



$C_8H_8O_3$: 152.15
Methyl 4-hydroxybenzoate
[*98-76-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Methyl Parahydroxybenzoate, when dried, contains not less than 98.0% and not more than 102.0% of $C_8H_8O_3$.

◆**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.◆

Identification **(1)** The melting point <2.60> of Methyl Parahydroxybenzoate is between 125°C and 128°C.

◆**(2)** Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Methyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆**(3)** Heavy metals <1.07>—Dissolve 1.0 g of Methyl Parahydroxybenzoate 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 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 0.10 g of Methyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample 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 Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot with the sample solution is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

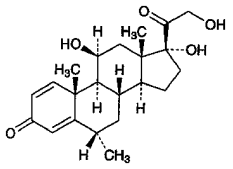
Assay Weigh accurately about 1.0 g of Methyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
$= 152.1$ mg of $C_8H_8O_3$

◆**Containers and storage** Containers—Well-closed containers.◆

Methylprednisolone

メチルプレドニゾロン



$C_{22}H_{30}O_5$: 374.47
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione
[*83-43-2*]

Methylprednisolone, when dried, contains not less than 96.0% and not more than 104.0% of $C_{22}H_{30}O_5$.

Description Methylprednisolone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in 1,4-dioxane, slightly soluble in ethanol (95) and in chloroform, and practically insoluble in water and in diethyl ether.

Melting point: 232 – 240°C (with decomposition).

Identification **(1)** Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

(2) Dissolve 10 mg of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Methylprednisolone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +79 – +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.2 g).

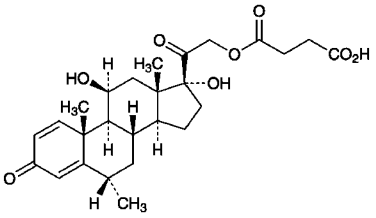
Assay Weigh accurately about 10 mg of Methylprednisolone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance A at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{22}H_{30}O_5 = A/400 \times 10{,}000$$

Containers and storage Containers—Tight containers.

Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル



$C_{26}H_{34}O_8$: 474.54
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate)
[*2921-57-5*]

Methylprednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{26}H_{34}O_8$.

Description Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Methylprednisolone Succinate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylprednisolone Succinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methylprednisolone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in ethanol (95), evaporating to dryness, and drying.

Optical rotation <*2.49*> $[\alpha]_D^{25}$: +99 – +103° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Methylprednisolone Succinate 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Methylprednisolone Succinate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 15 mg of Methylprednisolone Succinate in 5 mL of methanol, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than the peak of methylprednisolone succinate from sample solution is not larger than 1/2 times the peak area of methylprednisolone succinate from the standard solution, and the total area of the peaks other than the peak of methylprednisolone succinate is not larger than the peak area of methylprednisolone succinate 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.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of methylprednisolone succinate obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylprednisolone succinate is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g).

Assay Weigh accurately about 15 mg each of Methylprednisolone Succinate and Methylprednisolone Succinate RS, previously dried, dissolve separately in 5 mL of methanol, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methylprednisolone succinate to that of the internal standard.

$$\text{Amount (mg) of } C_{26}H_{34}O_8 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Methylprednisolone Succinate RS

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) (3 in 20,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of 0.05 mol/L disodium hydrogen phosphate TS to make a solution having pH 5.5. To 640 mL of this solution add 360 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylprednisolone succinate 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, methylprednisolone succinate 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylprednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Methylrosanilinium Chloride

Crystal Violet

メチルロザニリン塩化物

$C_{25}H_{30}ClN_3$: 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride ($C_{25}H_{30}ClN_3$)], calculated on the dried basis.

Description Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

It is soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

(2) Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

(3) To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

Purity (1) Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Methylrosanilinium Chloride 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) Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 7.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 1.5% (0.5 g).

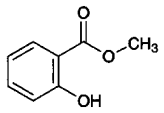
Assay Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate <*2.50*> the excess titanium (III) chloride with 0.05 mol/L ammonium iron (III) sulfate VS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 20.40 mg of $C_{25}H_{30}ClN_3$

Containers and storage Containers—Tight containers.

Methyl Salicylate

サリチル酸メチル



$C_8H_8O_3$: 152.15
Methyl 2-hydroxybenzoate
[*119-36-8*]

Methyl Salicylate contains not less than 98.0% of $C_8H_8O_3$.

Description Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor.

It is miscible with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Specific gravity d_{20}^{20}: 1.182 – 1.192

Boiling point: 219 – 224°C

Identification Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS: a purple color develops.

Purity (1) Acidity—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

(2) Heavy metals—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

Assay Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate <*2.50*> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS
= 76.08 mg of $C_8H_8O_3$

Containers and storage Containers—Tight containers.

Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

Method of preparation

Methyl Salicylate	40 mL
Capsicum Tincture	100 mL
d- or *dl*-Camphor	50 g
Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Compound Methyl Salicylate Spirit is a reddish yellow liquid, having a characteristic odor and a burning taste.

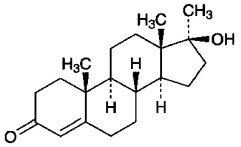
Identification **(1)** Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

(2) Shake thoroughly 0.5 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Dissolve 40 mg of methyl salicylate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and chloroform (4:1) to a distance of about 10 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *R*f value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Methyltestosterone

メチルテストステロン



$C_{20}H_{30}O_2$: 302.45
17β-Hydroxy-17α-methylandrost-4-en-3-one
[*58-18-4*]

Methyltestosterone, when dried, contains not less than 98.0% and not more than 102.0% of $C_{20}H_{30}O_2$.

Description Methyltestosterone occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Methyltestosterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyltestosterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methyltestosterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Methyltestosterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 163 – 168°C

Purity Related substances—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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 and diethylamine (19:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard.

$$\text{Amount (mg) of } C_{20}H_{30}O_2 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Methyltestosterone RS

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside 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°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone 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 methyltestosterone 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methyltestosterone Tablets

メチルテストステロン錠

Methyltestosterone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$: 302.45).

Method of preparation Prepare as directed under Tablets, with Methyltestosterone.

Identification To a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone according to the labeled amount, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methyltestosterone RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same Rf value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyltestosterone Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about 10 μg of methyltestosterone ($C_{20}H_{30}O_2$) per ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then add 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 standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.25*⟩.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2) = M_S \times A_T/A_S \times V'/V \times 1/10$$

M_S: Amount (mg) of Methyltestosterone RS

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 5 L as the dissolution medium, the dissolution rate in 30 minutes of a 10-mg tablet is not less than 75% and that in 60 minutes of a 25-mg tablet is not less than 70%.

Start the test with 1 tablet of Methyltestosterone Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of methyltestosterone ($C_{20}H_{30}O_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Methyltestosterone RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 10 hours, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 249 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of methyltestosterone } (C_{20}H_{30}O_2) = M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Methyltestosterone RS

C: Labeled amount (mg) of methyltestosterone ($C_{20}H_{30}O_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Methyltestosterone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone ($C_{20}H_{30}O_2$), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding 0.45 μm in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard.

Amount (mg) of methyltestosterone ($C_{20}H_{30}O_2$)
$= M_S \times Q_T/Q_S \times 5/4$

M_S: Amount (mg) of Methyltestosterone RS

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside 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°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

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

System suitability—

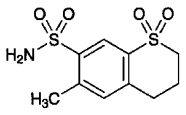
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Meticrane

メチクラン



$C_{10}H_{13}NO_4S_2$: 275.34
6-Methylthiochromane-7-sulfonamide 1,1-dioxide
[*1084-65-7*]

Meticrane, when dried, contains not less than 98.0% of $C_{10}H_{13}NO_4S_2$.

Description Meticrane occurs as white, crystals or crystalline powder. It is odorless and has a slight bitter taste.

It is freely soluble in dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Meticrane in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Meticrane, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Ammonium <*1.02*>—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Meticrane 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 <*1.11*>—Prepare the test solution with 1.0 g of Meticrane according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

Operating conditions 1—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside 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 water and acetonitrile (17:3).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 1—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 10 mg each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions 1, caffeine and meticrane 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 of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Operating conditions 2—

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

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

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.

Time span of measurement: About 10 times as long as the retention time of meticrane beginning after the solvent peak.
System suitability 2—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 20 mg each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate 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 of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

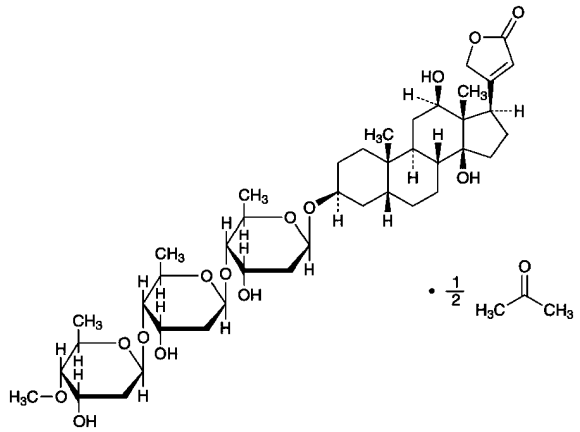
Assay Weigh accurately about 0.5 g of Meticrane, previously dried, dissolve in 50 mL of dimethylformamide, add 5 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 27.54 mg of $C_{10}H_{13}NO_4S_2$

Containers and storage Containers—Well-closed containers.

Metildigoxin

メチルジゴキシン



$C_{42}H_{66}O_{14}.\frac{1}{2}C_3H_6O$: 824.00

3*β*-[2,6-Dideoxy-4-*O*-methyl-*β*-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-*β*-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-*β*-D-*ribo*-hexopyranosyloxy]-12*β*,14-dihydroxy-5*β*-card-20(22)-enolide—acetone (2/1)
[*30685-43-9*, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of $C_{42}H_{66}O_{14}.\frac{1}{2}C_3H_6O$, calculated on the anhydrous basis.

Description Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

(2) Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

(3) Determine the absorption spectrum of a solution of Metildigoxin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Metildigoxin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Metildigoxin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Metildigoxin RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation ⟨*2.49*⟩ $[\alpha]_{546.1}^{20}$: +22.0 – +25.5° (1 g, calculated on the anhydrous basis, pyridine, 10 mL, 100 mm).

Purity **(1)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.5 g of Metildigoxin according to Method 3, and perform the test (not more than 4 ppm).

(2) Related substances—Dissolve 10 mg of Metildigoxin in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform 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 ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-butanone and chloroform (3:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Acetone Weigh accurately about 0.1 g of Metildigoxin, dissolve in exactly 2 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of acetone in a 50-mL volumetric flask containing about 10 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, then 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 sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩, 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 between 2.0% and 5.0%.

$$\text{Amount (\%) of acetone} = M_S/M_T \times Q_T/Q_S$$

M_S: Amount (g) of acetone
M_T: amount (g) of the sample

Internal standard solution—A solution of *t*-butanol in *N*,*N*-dimethylformamide (1 in 2000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column about 2 mm in inside diameter and 1 to 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature between 170°C and 230°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of acetone is about 2 minutes.
Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of acetone and *t*-butanol in this order with the resolution between these peaks being not less than 2.0.

Water ⟨*2.48*⟩ Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately 0.1 g each of Metildigoxin and Metildigoxin RS (separately, determine the water ⟨*2.48*⟩ in the same mammer as Metildigoxin), and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at 20 ± 0.5°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

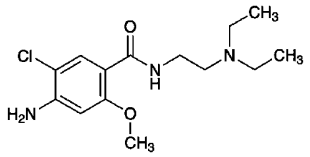
$$\text{Amount (mg) of } C_{42}H_{66}O_{14}\cdot\tfrac{1}{2}C_3H_6O = M_S \times A_T/A_S$$

M_S: Amount (mg) of Metildigoxin RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Metoclopramide

メトクロプラミド



$C_{14}H_{22}ClN_3O_2$: 299.80
4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide
[*364-62-5*]

Metoclopramide, when dried, contains not less than 99.0% of $C_{14}H_{22}ClN_3O_2$.

Description Metoclopramide occurs as white crystals or a crystalline powder, and is odorless.

It is freely soluble in acetic acid (100), soluble in methanol and in chloroform, sparingly soluble in acetic anhydride, in ethanol (95) and in acetone, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Dissolve 10 mg of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for Primary Aromatic Amines.

(2) Dissolve 10 mg of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff's TS: a reddish orange precipitate is produced.

(3) Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water to make 100 mL. To 1 mL of the solution add water to make 100 mL,

determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point ⟨*2.60*⟩ 146 – 149°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Metoclopramide as directed under 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 ⟨*1.11*⟩—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, exactly measured, with 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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-butanol and ammonia solution (28) (19:1) to a distance of about 10 cm. Dry the plate, first in air and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Metoclopramide, previously dried and accurately weighed, in 50 mL of acetic acid (100), add 5 mL of acetic anhydride, and warm for 5 minutes. Allow to cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform the blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of $C_{14}H_{22}ClN_3O_2$

Containers and storage Containers—Well-closed containers.

Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$: 299.80).

Method of preparation Prepare as directed under Tablets, with Metoclopramide.

Identification **(1)** To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide according to the labeled amount, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 270 nm and 274 nm, and between 306 nm and 310 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V mL of a solution so that each mL contains about 12 μg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of metoclopramide ($C_{14}H_{22}ClN_3O_2$)
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of metoclopramide for assay

Dissolution Being specified separately.

Assay Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

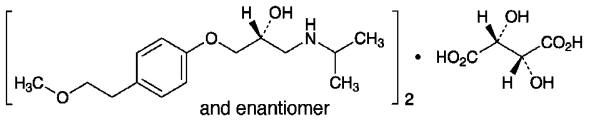
Amount (mg) of metoclopramide ($C_{14}H_{22}ClN_3O_2$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of metoclopramide for assay

Containers and storage Containers—Tight containers.

Metoprolol Tartrate

メトプロロール酒石酸塩



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$: 684.81
(2*RS*)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hemi-(2*R*,3*R*)-tartrate
[*56392-17-7*]

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$.

Description Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation $[\alpha]_D^{20}$: +7.0 – +10.0° (after drying, 1 g, water, 50 mL, 100 mm).

Identification **(1)** Determine the absorption spectrum of a solution of Metoprolol Tartrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metoprolol Tartrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Metoprolol Tartrate from a solution in acetone (23 in 1000), filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <*1.09*> (1) for tartrate.

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Metoprolol Tartrate 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) Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonia water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point with the sample solution is not more than three spots, and they are not more intense than the spot with the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Metoprolol Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.24 mg of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$: 684.81).

Method of preparation Prepare as directed under Tablets, with Metoprolol Tartrate.

Identification To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate according to the labeled amount, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add ethanol (95) to make exactly V' so that each mL contains about 0.1 mg of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 5 mL of water, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using ethanol (95) as the blank.

Amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$
$= M_S \times A_T/A_S \times V'/V \times 1/5$

M_S: Amount (mg) of metoprolol tartrate for assay

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Metoprolol Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Metoprolol Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$ according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 200 mL. Pipet 8 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of metoprolol.

Dissolution rate (%) with respect to the labeled amount of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of metoprolol tartrate for assay
C: Labeled amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$ 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 number of theoretical plates and the symmetry factor of the peak of metoprolol are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metoprolol is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Metoprolol Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.12 g of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$, add 60 mL of a mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) and exactly 10 mL of the internal standard solution, shake for 15 minutes, and add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.12 g of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 60 mL of the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, then add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of metoprolol to that of the internal standard.

Amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6)$
$= M_S \times Q_T/Q_S$

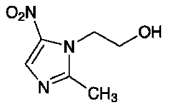
M_S: Amount (mg) of metoprolol tartrate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 274 nm).
Column: A stainless steel column 4.6 mm in inside 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: Dissolve 14.0 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust to pH 3.2 with diluted perchloric acid (17 in 2000). To 750 mL of this solution add 250 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of metoprolol 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, metoprolol 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metoprolol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Metronidazole

メトロニダゾール



$C_6H_9N_3O_3$: 171.15
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol
[*443-48-1*]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of $C_6H_9N_3O_3$.

Description Metronidazole occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

Identification **(1)** Determine the absorption spectrum of a

solution of Metronidazole in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metronidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 159 – 163°C.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Metronidazole 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) 2-Methyl-5-nitroimidazol—Dissolve 0.10 g of Metronidazole in acetone to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetone to make exactly 20 mL, then pipet 5 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 Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of *p*-naphtholbenzein TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.12 mg of $C_6H_9N_3O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$: 171.15).

Method of preparation Prepare as directed under Tablets, with Metronidazole.

Identification **(1)** To an amount of powdered Metronidazole Tablets, equivalent to 0.1 g of Metronidazole according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand for 30 minutes with occasional stirring. Then, shake vigorously, and centrifuge a part of this solution. To 1 mL of the supernatant liquid add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 275 nm and 279 nm.

(2) Shake vigorously a quantity of powdered Metronidazole Tablets, equivalent to 0.20 g of Metronidazole according to the labeled amount, with 20 mL of acetone for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.10 g of metronidazole in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate immediately with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spots obtained from the sample solution and the standard solution is the same.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metronidazole Tablets add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 25 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter the solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

Amount (mg) of metronidazole ($C_6H_9N_3O_3$)
$= M_S \times A_T/A_S \times 10$

M_S: Amount (mg) of metronidazole for assay

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Metronidazole Tablets is not less than 70%.

Start the test with 1 tablet of Metronidazole Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 *V* mL of the subsequent filtrate, add water to make exactly *V'* mL so that each mL contains about 11 μg of metronidazole ($C_6H_9N_3O_3$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of metronidazole for assay, previously dried in vacuum with silica gel for 24 hours, 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 320 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of metronidazole ($C_6H_9N_3O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of metronidazole for assay
C: Labeled amount (mg) of metronidazole ($C_6H_9N_3O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole ($C_6H_9N_3O_3$), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of metronidazole.

Amount (mg) of metronidazole ($C_6H_9N_3O_3$)
$= M_S \times A_T/A_S \times 10$

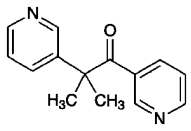
M_S: Amount (mg) of metronidazole for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 320 nm).
Column: A stainless steel column 4.6 mm in inside 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 water and methanol (4:1).
Flow rate: Adjust the flow rate so that the retention time of metronidazole 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 theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 1.0%.

Containers and storage Containers—Tight containers.

Metyrapone

メチラポン



$C_{14}H_{14}N_2O$: 226.27
2-Methyl-1,2-di(pyridin-3-yl)propan-1-one
[*54-36-4*]

Metyrapone, when dried, contains not less than 98.0% of $C_{14}H_{14}N_2O$.

Description Metyrapone occurs as a white to pale yellow, crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

Identification **(1)** Mix 5 mg of Metyrapone with 10 mg of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 50 – 54°C.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Metyrapone 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 <*1.11*>—Prepare the test solution with 1.0 g of Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Metyrapone in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample 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 and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

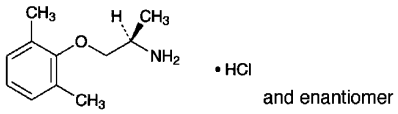
Assay Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 40 mL of acetic anhydride, and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.31 mg of $C_{14}H_{14}N_2O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Mexiletine Hydrochloride

メキシレチン塩酸塩



$C_{11}H_{17}NO.HCl$: 215.72
(1*RS*)-2-(2,6-Dimethylphenoxy)-1-methylethylamine monohydrochloride
[*5370-01-4*]

Mexiletine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{11}H_{17}NO.HCl$.

Description Mexiletine Hydrochloride occurs as a white powder.

It is freely soluble in water and in ethanol (95), slightly soluble in acetonitrile, and practically insoluble in diethyl ether.

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

Identification **(1)** Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Mexiletine Hydrochloride from ethanol (95), filter, dry the crystals, and repeat the test on the crystals.

(3) A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨1.09⟩ (2) for chloride.

pH ⟨2.54⟩ Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

Melting point ⟨2.60⟩ 200 – 204°C.

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

(2) Heavy Metals ⟨1.07⟩—Proceed with 2.0 g of Mexiletine 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) Related substances—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each peak area of the peaks other than the peak of mexiletine from the sample solution is not larger than the peak area of mexiletine from 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 mexiletine obtained from 20 μL of the standard solution is between 5 mm and 10 mm.

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

Loss on drying ⟨2.41⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of mexiletine to that of the internal standard, respectively.

Amount (mg) of $C_{11}H_{17}NO.HCl$
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Mexiletine Hydrochloride RS

Internal standard solution—A solution of phenetylamine hydrochloride in the mobile phase (3 in 5000).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (about 7 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogenphosphate dihydrate in 600 mL of

water, and add 420 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mexiletine is about 6 minutes.

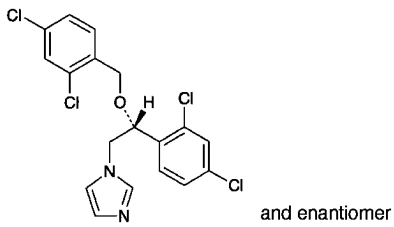
Selection of column: Proceed with 20 μL of the standard solution under the above conditions, and calculate the resolution. Use a column giving elution of the internal standard and mexiletine in this order with the resolution between these peaks being not less than 9.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Miconazole

ミコナゾール



and enantiomer

$C_{18}H_{14}Cl_4N_2O$: 416.13

1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole

[*22916-47-8*]

Miconazole, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O$.

Description Miconazole occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), soluble in diethyl ether, and practically insoluble in water.

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Miconazole in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Miconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 84 – 87°C.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Miconazole 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 <*1.11*>—Prepare the test solution with 1.0 g of Miconazole according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Miconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 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 <*2.03*>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 60%, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

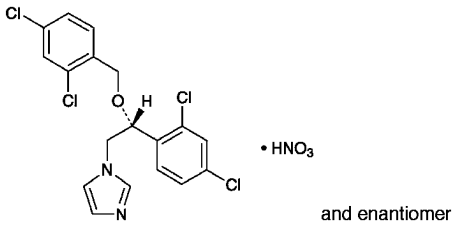
Assay Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of *p*-naphtholbenzein TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.61 mg of $C_{18}H_{14}Cl_4N_2O$

Containers and storage Containers—Tight containers.

Miconazole Nitrate

ミコナゾール硝酸塩



and enantiomer

$C_{18}H_{14}Cl_4N_2O.HNO_3$: 479.14

1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole mononitrate

[*22832-87-7*]

Miconazole Nitrate, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O.HNO_3$.

Description Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone and in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

Identification **(1)** To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (1 in 2500) as directed under

Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <1.04> (2): a green color appears.

(4) A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <1.09> for nitrate.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide 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 *N*,*N*-dimethylformamide to make 50 mL (not more than 0.09%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Miconazole Nitrate 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).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 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 <2.03>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *n*-hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

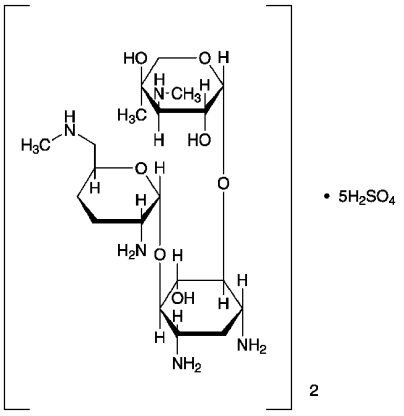
Assay Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Micronomicin Sulfate

ミクロノマイシン硫酸塩



$(C_{20}H_{41}N_5O_7)_2.5H_2SO_4$: 1417.53
2-Amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine hemipentasulfate
[*52093-21-7*, Micronomicin]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.

It contains not less than 590 μg (potency) and not more than 660 μg (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micronomicin ($C_{20}H_{41}N_5O_7$: 463.57).

Description Micronomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their *R*f values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation <2.49> $[\alpha]_D^{20}$: +110 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Micronomicin 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).

(3) Related substances—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 ⟨*2.03*⟩. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ Not more than 10.0% (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.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

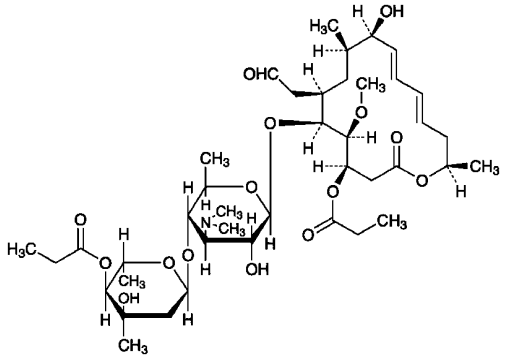
(iii) Standard solutions—Weigh accurately an amount of Micronomicin Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Midecamycin

ミデカマイシン



$C_{41}H_{67}NO_{15}$: 813.97

(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[2,6-Dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-*β*-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [*35457-80-8*]

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces mycarofaciens*.

It contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin ($C_{41}H_{67}NO_{15}$).

Description Midecamycin occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Midecamycin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 153 – 158°C.

Purity Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Midecamycin 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 ⟨*2.41*⟩ Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics

<4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

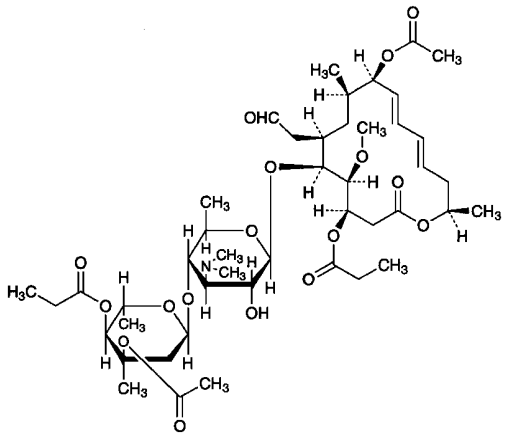
(iii) Standard solutions—Weigh accurately an amount of Midecamycin RS, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use 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 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Midecamycin Acetate

ミデカマイシン酢酸エステル



$C_{45}H_{71}NO_{17}$: 898.04

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-9-Acetoxy-5-[3-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propioyloxyhexadeca-10,12-dien-15-olide [*55881-07-7*]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass of midecamycin acetate ($C_{45}H_{71}NO_{17}$).

Description Midecamycin Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Midecamycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of dried Midecamycin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin 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).

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate RS, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°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 4.5 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin Acetate, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine ($C_{11}H_{12}N_2O$: 188.23) and not less than 8.6% and not more than 9.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

Description Migrenin occurs as a white powder or crystalline powder. It is odorless and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and slightly soluble in diethyl ether.

The pH of a solution of Migrenin (1 in 10) is between 3.0 and 4.0.

It is affected by moisture and light.

Identification **(1)** To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the residue over a vessel containing 3 drops of ammonia TS: a red-purple color develops, disappearing on the addition of 2 to 3 drops of sodium hydroxide TS.

(3) A solution of Migrenin (1 in 10) responds to the Qualitative Tests <*1.09*> for citrate.

Melting point <*2.60*> 104 – 110°C.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Migrenin 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 <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay **(1)** Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate <*2.50*> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS
= 9.411 mg of $C_{11}H_{12}N_2O$

(2) Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine RS, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of caffeine to that of the internal standard.

Amount (mg) of caffeine ($C_8H_{10}N_4O_2$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Caffeine RS

Internal standard solution—A solution of ethenzamide in chloroform (1 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

System suitability—

System performance: Dissolve 0.9 g of antipyrine and 0.09 g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the resolution between these peaks being not less than 1.5.

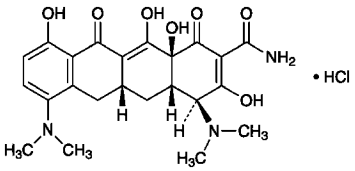
System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Minocycline Hydrochloride

ミノサイクリン塩酸塩



$C_{23}H_{27}N_3O_7$.HCl: 493.94
(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride
[*13614-98-7*]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline.

It contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Description Minocycline Hydrochloride occurs as a yellow crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 1.0 g of Minocycline Hydrochloride in 100 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity **(1)** A solution of Minocycline Hydrochloride (1 in 100) is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.06.

(2) Heavy metals <*1.07*>—Proceed with 0.5 g of Minocycline Hydrochloride 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).

(3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

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 minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

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

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

Water <*2.48*> Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of minocycline of these solutions.

$$\text{Amount [}\mu\text{g (potency)] of minocycline } (C_{23}H_{27}N_3O_7) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Minocycline Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

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

Column temperature: A constant temperature of about

25°C.

Mobile phase: Adjust to pH 6.5 of a mixture of a solution of ammonium oxalate monohydrate (7 in 250), *N*,*N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) with tetrabutylammonium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes.

System suitability—

System performance: Dissolve 50 mg of Minocycline Hydrochloride in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline 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 of the standard solution under the above operating conditions, the relative standard deviation of peak areas of minocycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Minocycline Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation Prepare as directed under Injections, with Minocycline Hydrochloride.

Description Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown powder or flakes.

Identification Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

pH ⟨*2.54*⟩ The pH of a solution, prepared by dissolving an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, in 10 mL of water is 2.0 to 3.5.

Purity Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, dissolve in the mobile phase to make 100 mL. To 25 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epiminocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

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

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

Water ⟨*2.48*⟩ Weigh accurately the mass of the content of one container of Minocycline Hydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and perform the test with exactly 1 mL of this solution as directed in the Volumetric titration (back titration): not more than 3.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.25 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Minocycline Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mobile phase to make exactly 100 mL. Pipet 25 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

$$\text{Amount [mg (potency)] of minocycline } (C_{23}H_{27}N_3O_7) = M_S \times A_T/A_S \times 4$$

M_S: Amount [mg (potency)] of Minocycline Hydrochlo-

ride RS

Containers and storage Containers—Hermetic containers.

Minocycline Hydrochloride Tablets

ミノサイクリン塩酸塩錠

Minocycline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of Minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation Prepare as directed under Tablets, with Minocycline Hydrochloride.

Identification To a quantity of powdered Minocycline Hydrochloride Tablets, equivalent to 10 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 625 mL of a solution of hydrochloric acid in methanol (19 in 20,000), shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

Purity Related substances—Conduct this procedure rapidly after preparation of the sample solution. Powder not less than 5 Minocycline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 50 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 60 mL of the mobile phase, shake vigorously, and add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the amount of the peak of epiminocycline, having the relative retention time of about 0.83 with respect to minocycline, 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.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: To 2 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline from 20 μL of the solution for system suitability test.

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

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

Water <*2.48*> Not more than 12.0% (0.5 g of powdered Minocycline Hydrochloride Tablets, volumetric titration, back titration).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Minocycline Hydrochloride Tablets add 60 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg (potency) of Minocycline Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount [mg (potency)] of Minocycline Hydrochloride RS

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Minocycline Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Minocycline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 9 μg (potency) of Minocycline Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 348 nm.

Dissolution rate (%) with respect to the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount [mg (potency)] of Minocycline Hydrochloride RS

C: Labeled amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$) in 1 tablet

Assay To a number of Minocycline Hydrochloride Tablets, equivalent to about 1 g (potency) of Minocycline Hydrochloride, add 120 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly 200 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

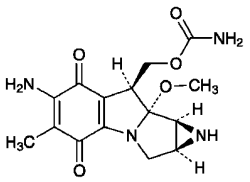
Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$) = $M_S \times A_T/A_S \times 40$

M_S: Amount [mg (potency)] of Minocycline Hydrochloride RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Mitomycin C

マイトマイシン C



$C_{15}H_{18}N_4O_5$: 334.33
(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-4,7-dioxo-8a-methoxy-5-methyl-1,1a,2,8,8a,8b-hexahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-ylmethyl carbamate
[*50-07-7*]

Mitomycin C is a substance having antitumor activity produced by the growth of *Streptomyces caespitosus*.

It contains not less than 970 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C ($C_{15}H_{18}N_4O_5$).

Description Mitomycin C occurs as blue-purple, crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not larger than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not larger than 3 times the peak area of mitomycin C from the standard solution.

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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 30°C.

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 30	100 → 0	0 → 100
30 – 45	0	100

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of mitomycin C beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

Loss on drying <*2.41*> Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Mitomycin C and Mitomycin C RS, equivalent to about 25 mg (potency), dissolve each in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the fol-

lowing conditions, and determine the peak areas, A_T and A_S, of mitomycin C.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{18}N_4O_5 = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Mitomycin C RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 365 nm).

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mitomycin C is about 7 minutes.

System suitability—

System performance: Dissolve about 25 mg of Mitomycin C RS and about 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N*,*N*-dimethylacetamide. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 1.0%.

Containers and storage Containers—Tight containers.

Mitomycin C for Injection

注射用マイトマイシン C

Mitomycin C for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of mitomycin C ($C_{15}H_{18}N_4O_5$: 334.33).

Method of preparation Prepare as directed under Injections, with Mitomycin C.

Description Mitomycin C for Injection occurs as a blue-purple powder.

Identification Dissolve an amount of Mitomycin C for Injection, equivalent to 2 mg (potency) of Mitomycin C according to the labeled amount, in 200 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 216 nm and 220 nm, and between 362 nm and 366 nm.

pH ⟨*2.54*⟩ The pH of a solution, prepared by dissolving 0.25 g of Mitomycin C for Injection in 20 mL of water, is 5.5 to 8.5.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.4 g, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 container of Mitomycin C for Injection add exactly V mL of *N*,*N*-dimethylacetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C RS, add *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

$$\text{Amount [mg (potency)] of mitomycin C } (C_{15}H_{18}N_4O_5) = M_S \times A_T/A_S \times V/50$$

M_S: Amount [mg (potency)] of Mitomycin C RS

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Mitomycin C for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Mitomycin C, add exactly 20 mL of *N*,*N*-dimethylacetamide, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount of Mitomycin C RS, equivalent to about 25 mg (potency), dissolve in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

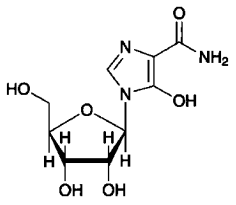
$$\text{Amount [mg (potency)] of mitomycin C } (C_{15}H_{18}N_4O_5) = M_S \times A_T/A_S \times 2/5$$

M_S: Amount [mg (potency)] of Mitomycin C RS

Containers and storage Containers—Hermetic containers.

Mizoribine

ミゾリビン



$C_9H_{13}N_3O_6$: 259.22

5-Hydroxy-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide [*50924-49-7*]

Mizoribine contains not less than 98.0% and not more than 102.0% of $C_9H_{13}N_3O_6$, calculated on the anhydrous basis.

Description Mizoribine occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Mizoribine (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mizoribine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mizoribine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Mizoribine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -25 – $-27°$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mizoribine 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 0.10 g of Mizoribine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 50 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 exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the areas of the peaks other than mizoribine obtained from the sample solution are not larger than the mizoribine peak area from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

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

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 mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% 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, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

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 mizoribine is not more than 2.0%.

Water <*2.48*> Not more than 0.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Mizoribine, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Mizoribine RS (separately determine the water <*2.48*> using the same manner as Mizoribine), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of mizoribine, A_T and A_S, of both solutions.

$$\text{Amount (mg) of } C_9H_{13}N_3O_6 = M_S \times A_T/A_S \times 10$$

M_S: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4.6 mm in inside 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: Diluted phosphoric acid (1 in 1500).

Flow rate: Adjust the flow rate so that the retention time of mizoribine 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 the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

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 mizoribine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Mizoribine Tablets

ミゾリビン錠

Mizoribine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of mizoribine ($C_9H_{13}N_3O_6$: 259.22).

Method of preparation Prepare as directed under Tablets, with Mizoribine.

Identification To a quantity of powdered Mizoribine Tablets, equivalent to 0.1 g of Mizoribine according to the labeled amount, add 5 mL of water, shake, filter, and use the filtrate as the sample solution. Separately, dissolve 20 mg of Mizoribine RS in 1 mL of water, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-Layer Chromatography <*2.03*>. Spot 1 μL each of the sample solu-

tion and standard solution on a plate of silica gel for thin-layer chromatography. Then develop the plate with a mixture of methanol, ammonia solution (28) and 1-propanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the principal spot from the sample solution and the spot from the standard solution show a red-brown color and the same *R*f value.

Purity Related substances—To a quantity of powdered Mizoribine Tablets, equivalent to 0.10 g of Mizoribine according to the labeled amount, add 30 mL of the mobile phase, shake, then add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.5 μm and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 with respect to mizoribine, obtained from the sample solution is not larger than the peak area of mizoribine from the standard solution, and the area of the peak other than mizoribine and other than the peak mentioned above is not larger than 2/5 times the peak area of mizoribine from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Mizoribine.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% 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, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

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 mizoribine is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mizoribine Tablets add 50 mL of water, shake until the tablet is disintegrated, and add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5 μg of mizoribine ($C_9H_{13}N_3O_6$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine RS (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the 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 279 nm of the sample solution and standad solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount of mizoribine } (C_9H_{13}N_3O_6) = M_S \times A_T/A_S \times V'/V \times 1/50$$

M_S: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mizoribine Tablets is not less than 80%.

Start the test with 1 tablet of Mizoribine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 14 μg of mizoribine ($C_9H_{13}N_3O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mizoribine RS (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 1 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 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of mizoribine } (C_9H_{13}N_3O_6) = M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

C: Labeled amount (mg) of mizoribine ($C_9H_{13}N_3O_6$) in 1 tablet

Assay Weigh accurately not less than 20 Mizoribine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of mizoribine ($C_9H_{13}N_3O_6$), add 50 mL of water and shake, then add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine RS (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the 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 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of mizoribine } (C_9H_{13}N_3O_6) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Morphine and Atropine Injection

モルヒネ·アトロピン注射液

Morphine and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

Method of preparation

Morphine Hydrochloride Hydrate	10 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a significant quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Morphine and Atropine Injection is a clear, colorless liquid.

It is gradually colored by light.

pH: 2.5 – 5.0

Identification To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same *R*f value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

Extractable volume <*6.05*> It meets the requirement.

Assay (1) Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= M_S \times Q_T/Q_S \times 1.168$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. 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 morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate RS (separately determine the loss on drying <*2.41*> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 50 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
$= M_S \times Q_T/Q_S \times 1/25 \times 1.027$

M_S: Amount (mg) of Atropine Sulfate RS, calculated on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 12,500).

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

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

Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.

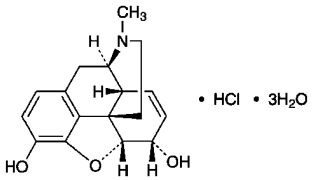
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物



$C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84

(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate

[*6055-06-7*]

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl$: 321.80), calculated on the anhydrous basis.

Description Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

It gradually becomes yellow-brown by light.

Identification **(1)** Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Morphine Hydrochloride in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Morphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −111 – −116° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.40 g of Morphine Hydrochloride Hydrate in 10 mL of water: the solution is clear. When perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, the absorbance at 420 nm is not more than 0.12.

(2) Sulfate <*1.14*>—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Related substances—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) 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 <*2.03*>. Spot 10 μL each of the sample solution and 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 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 13 – 15% (0.1 g, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), mix, and titrate <*2.50*> 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
= 32.18 mg of $C_{17}H_{19}NO_3.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Morphine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochlo-

ride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84).

Method of preparation Prepare as directed under Injections, with Morphine Hydrochloride Hydrate.

Description Morphine Hydrochloride Injection is a clear, colorless or pale yellow-brown liquid.

It gradually becomes yellow-brown by light.

pH: 2.5 – 5.0

Identification Take a volume of Morphine Hydrochloride Injection, equivalent to 0.04 g of Morphine Hydrochloride Hydrate according to the labeled amount, add water to make 20 mL, and use this solution as the sample solution. To 5 mL of the sample solution add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm. And to 5 mL of the sample solution add dilute sodium hydroxide TS to make 100 mL, and determine the absorption spectrum: it exhibits a maximum between 296 nm and 300 nm.

Bacterial endotoxins <*4.01*> Less than 1.5 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= M_S \times Q_T/Q_S \times 4 \times 1.168$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 retention time of morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

Morphine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84).

Method of preparation Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

Identification Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), disperse the tablet into a small particles using ultrasonic waves, then treat with ultrasonic waves for 15 minutes with occasional stirring, and add water to make V mL so that each mL contains about 0.4 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= M_S \times Q_T/Q_S \times V/50 \times 1.168$

M_S: Amount (mg) of morphine hydrochloride for assay,

calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Morphine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Morphine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of morphine hydrochloride for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Morphine Hydrochloride Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of morphine in each solution.

Dissolution rate (%) with respect to the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= M_S \times A_T/A_S \times 1/C \times 36 \times 1.168$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$) 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 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of morphine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 2.0%.

Assay Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= M_S \times Q_T/Q_S \times 1.168$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 morphine 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, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

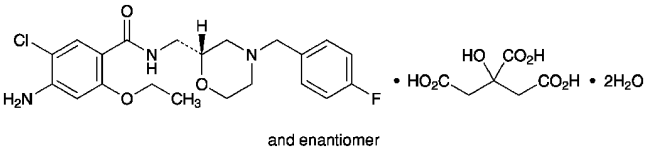
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mosapride Citrate Hydrate

モサプリドクエン酸塩水和物



and enantiomer

$C_{21}H_{25}ClFN_3O_3.C_6H_8O_7.2H_2O$: 650.05

4-Amino-5-chloro-2-ethoxy-*N*-{[(2*RS*)-4-(4-fluorobenzyl)morpholin-2-yl]methyl}benzamide monocitrate dihydrate

[*636582-62-2*]

Mosapride Citrate Hydrate contains not less than 98.5% and not more than 101.0% of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$: 614.02), calculated on the anhydrous basis.

Description Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Mosapride Citrate Hydrate in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Mosapride Citrate Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mosapride Citrate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mosapride Citrate Hydrate in *N*,*N*-dimethylformamide (1 in 10) responds to the Qualitative Tests <*1.09*> (1) for citrate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mosapride Citrate Hydrate 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).

(2) Related substances—Dissolve 0.10 g of Mosapride Citrate Hydrate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.47 with respect to mosapride from the sample solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than the peak mentioned above from the sample solution is not larger than the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than the peak of mosapride from the sample solution is not larger than 5 times the peak area of mosapride from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 4.0 with dilute hydrochloric acid, and add water to make 1000 mL.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 35	80 → 45	20 → 55

Flow rate: 1.0 mL per minute.

Time span of measurement: Beginning after the solvent peak to 35 minutes after injection.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 μL of this solution is equivalent to 15 to 25% of that of mosapride 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, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.

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 mosapride is not more than 5.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> 5.0 – 6.5% (0.5 g, volumetric titration, back titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 61.40 mg of $C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Mosapride Citrate Powder

モサプリドクエン酸塩散

Mosapride Citrate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$: 614.02).

Method of preparation Prepare as directed under Granules or Powders, with Mosapride Citrate Hydrate.

Identification **(1)** Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima be-

tween 271 nm and 275 nm and between 306 nm and 310 nm.

Purity Related substances—Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, moisten with 1 mL of water, then add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the two peaks, having the relative retention time of about 0.60 and about 0.85 with respect to mosapride obtained from the sample solution, is not larger than the peak area of mosapride from the standard solution, the area of other than the peak of mosapride and the peaks mentioned above is not larger than 2/5 times the peak area of mosapride from the standard solution, and the total area of the peak other than mosapride is not larger than 2 times the peak area of mosapride from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 40	85 – 45	15 – 55

Time span of measurement: For 40 minutes after sample injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained with 10 μL of this solution is equivalent to 3.0 to 5.0% 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 mosapride are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: the powder in single-unit container meets the requirement of the Content uniformity test.

To the total content of 1 container of Mosapride Citrate Powder add 5 mL of water, and shake. Then, add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 20 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Powder is not less than 70%.

Start the test with an amount of Mosapride Citrate Powder, equivalent to about 2.5 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately determine the water <*2.48*> in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, and determine the peak areas, A_T and A_S, of mosapride of both solutions.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 9$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis
M_T: Amount (g) of sample
C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust to pH 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

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 mosapride are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of mosapride is not more than 2.0%.

Assay Powder Mosapride Citrate Powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), moisten with 2 mL of water, add 70 mL of methanol, shake for 20 minutes, then add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately determine the water <2.48> in the same manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. To 2 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 the sample solution and the standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) $= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Mosapride Citrate Tablets

モサプリドクエン酸塩錠

Mosapride Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$: 614.02).

Method of preparation Prepare as directed under Tablets, with Mosapride Citrate Hydrate.

Identification **(1)** To an amount of powdered Mosapride Citrate Tablets, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 271 nm and 275 nm, and between 306 nm and 310 nm.

Purity Related substances—Powder not less than 20 tablets of Mosapride Citrate Tablets. Moisten a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, with 1 mL of water. Add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL. Pipet 2 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks having the relative retention times of about 0.60 and about 0.85 with respect to mosapride from the sample solution is not larger than the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than those mentioned above from the sample solution is not larger than 2/5 times the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than mosapride from the sample solution is not larger than 2 times the peak area of mosapride from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 40	85 → 45	15 → 55

Time span of measurement: Beginning after the solvent peak to 40 minutes after injection.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained from 10 μL of this solution is equivalent to 3.0 to 5.0% of that of mosapride 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 the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mosapride Citrate Tablets add 5 mL of water, and shake well to disintegrate. Add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 20 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) $= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of

Mosapride Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Mosapride Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 2.8 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately, determine the water <2.48> in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of mosapride of both solutions.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

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 mosapride are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Mosapride Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately, determine the water <2.48> in the manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 273 nm.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
$= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Freeze-dried Live Attenuated Mumps Vaccine

乾燥弱毒生おたふくかぜワクチン

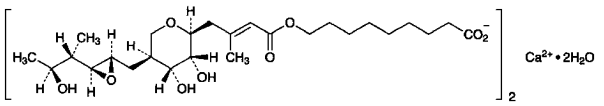
Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

Mupirocin Calcium Hydrate

ムピロシンカルシウム水和物



$C_{52}H_{86}CaO_{18}.2H_2O$: 1075.34

Monocalcium bis[9-((2*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxy-3,4,5,6-tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy)nonanoate] dihydrate

[*115074-43-6*]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Pseudomonas fluorescens*.

It contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin ($C_{26}H_{44}O_9$: 500.62).

Description Mupirocin Calcium Hydrate occurs as a white powder and has a bitter taste.

It is freely soluble in methanol and slightly soluble in water and in ethanol (95).

Identification **(1)** To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of *N*,*N*′-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchorate hexahydrate-ethanol TS to the solution, and shake: a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1708 cm^{-1}, 1648 cm^{-1}, 1558 cm^{-1}, 1231 cm^{-1}, 1151cm^{-1} and 894 cm^{-1}.

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests <*1.09*> (3) for calcium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -16 – $-20°$ (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of the sample solution (1), add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the sample solution (2). Preserve these sample solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related substance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

$$\text{Amount (\%) of principal related substance} = \frac{A_i}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \dfrac{A \times 100}{A + A_m}}$$

$$\text{Total amount (\%) of related substances} = \frac{A}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \dfrac{A \times 100}{A + A_m}}$$

A: Total peak areas other than of the solvent and mupirocin from the sample solution (1)

A_i: Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)

A_m: A value of 50 times of peak area of mupirocin from the sample solution (2)

P: Potency per mg obtained from the assay

Operating conditions—

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

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

System suitability—

Test for required detection: Pipet 1 mL of the sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the sample solution (2).

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

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

(2) Inorganic salt from manufacturing process—Being specified separately.

Water <*2.48*> Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of mupirocin of each solution.

$$\text{Amount [}\mu\text{g (potency)] of mupirocin } (C_{26}H_{44}O_9) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Mupirocin Lithium RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of mupirocin is about 12.5 minutes.

System suitability—

System performance: Dissolve about 20 mg of Mupirocin Lithium RS and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Mupirocin Calcium Ointment

ムピロシンカルシウム軟膏

Mupirocin Calcium Ointment is an oily ointment preparation.

Mupirocin Calcium Ointment contains not less than 95.0% and not more than 105.0% of the labeled potency of mupirocin ($C_{26}H_{44}O_9$: 500.62).

Method of preparation Prepare as directed under Ointments, with Mupirocin Calcium Hydrate.

Identification To an amount of Mupirocin Calcium Ointment, equivalent to 10 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of water, and warm on a water bath at 60°C for 10 minutes while occasional shaking. After cooling, filter, and 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 <*2.24*>: it exhibits a maximum between 220 nm and 224 nm.

Purity Related substances—To an amount of Mupirocin Calcium Ointment, equivalent to 50 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. Then, add 5 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the area of the peak other than mupirocin obtained from the sample solution and the peak area of mupirocin from the standard solution by the automatic integration method. Calculate the amount of each related substance using the following equation: the amount of the related substance having the relative retention time of about 0.7 to mupirocin is not more than 4.0%, the amount of the related substance other than that is not more than 1.5 %, and the total amount of the related substances is not more than 6.0%.

$$\text{Amount (\%) of each related substance} = A/(\Sigma A + A_m) \times 100$$

A: Peak area of each related substance obtained from the sample solution.

ΣA: Total area of the peaks other than mupirocin obtained from the sample solution.

A_m: Amount of 50 times the peak area of mupirocin 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 under Mupirocin Calcium Hydrate.

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained with 20 μL of this solution is equivalent to 4 to 6% of that with 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay under Mupirocin Calcium Hydrate.

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

Assay Weigh accurately an amount of Mupirocin Calcium Ointment, equivalent to about 2 mg (potency) of Mupirocin Calcium Hydrate, add exactly 10 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. To this solution add exactly 10 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mupirocin Calcium Hydrate.

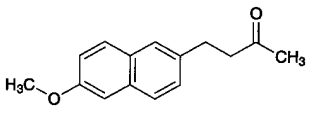
$$\text{Amount [mg (potency)] of mupirocin } (C_{26}H_{44}O_9) = M_S \times A_T/A_S \times 1/10$$

M_S: Amount [mg (potency)] of Mupirocin Lithium RS

Containers and storage Containers—Tight containers.

Nabumetone

ナブメトン



$C_{15}H_{16}O_2$: 228.29

4-(6-Methoxynaphthalen-2-yl)butan-2-one

[*42924-53-8*]

Nabumetone contains not less than 98.0% and not more than 101.0% of $C_{15}H_{16}O_2$, calculated on the anhydrous basis.

Description Nabumetone occurs as white to yellowish white crystals or a crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Nabumetone in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nabumetone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nabumetone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nabumetone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 79 – 84°C.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Nabumetone 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 20 mg of Nabumetone in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetonitrile to make exactly 50 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area of the related substance G obtained from the sample solution is not larger than 3/5 times the peak area of nabumetone from the standard solution, and each peak area other than nabumetone and the related substance G is not larger than 1/5 times the peak area of nabumetone from the standard solution. Furthermore, the total area of the peaks other than nabumetone is not larger than 1.6 times the peak area of nabumetone from the standard solution. For these calculations, use each peak area of the related substances A, B, C, D, E, F and G, which are having the relative retention time of about 0.73, 0.85, 0.93, 1.2, 1.9, 2.6 and 2.7 with respect to nabumetone, after multiplying by their relative response factors, 0.12, 0.94, 0.25, 0.42, 1.02, 0.91 and 0.1, respectively.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of water and acetic acid (100) (999:1).

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (7:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12	60	40
12 – 28	60 → 20	40 → 80

Flow rate: 1.3 mL per minute.

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

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nabumetone obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 5.0%.

Water <2.48> Not more than 0.2% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Nabumetone and Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), dissolve them in acetonitrile to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of nabumetone, A_T and A_S, from each solution.

$$\text{Amount (mg) of } C_{15}H_{16}O_2 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: To 600 mL of a mixture of water and acetic acid (100) (999:1) add 400 mL of a mixture of acetonitrile and tetrahydrofuran (7:3).

Flow rate: Adjust the flow rate so that the retention time of nabumetone 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 the symmetry factor of the peak of nabumetone are not less than 6000 and not more than 1.5, respectively.

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

Containers and storage Containers—Tight containers.

Nabumetone Tablets

ナブメトン錠

Nabumetone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nabumetone ($C_{15}H_{16}O_2$: 228.29).

Method of preparation Prepare as directed under Tablets, with Nabumetone.

Identification To a quantity of powdered Nabumetone Tablets, equivalent to 80 mg of Nabumetone according to the labeled amount, add 50 mL of methanol, shake for 10 minutes and centrifuge the solution. To 1 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 259 nm and 263 nm, between 268 nm and 272 nm, between 316 nm and 320 nm, and between 330 nm and 334 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80 (dissolving 3 g of polysorbate 80 in water to make 100 mL) as the dissolution medium, the dissolution rate in 60 minutes of Nabumetone Tablets is not less than 70%.

Start the test with 1 tablet of Nabumetone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 a solution, prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL, to make exactly V' mL so that each mL contains about 89 μg of nabumetone ($C_{15}H_{16}O_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 331 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL as the blank.

Dissolution rate (%) with respect to the labeled amount of nabumetone ($C_{15}H_{16}O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 360$

M_S: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis
C: Labeled amount (mg) of nabumetone ($C_{15}H_{16}O_2$) in 1 tablet

Assay Weigh accurately not less than 20 tablets of Nabumetone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of nabumetone ($C_{15}H_{16}O_2$), add 10 mL of water and shake, add 40 mL of methanol, shake for 30 minutes, and then add methanol to make exactly 100 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nabumetone to that of the internal standard.

Amount (mg) of nabumetone ($C_{15}H_{16}O_2$)
$= M_S \times Q_T/Q_S \times 5$

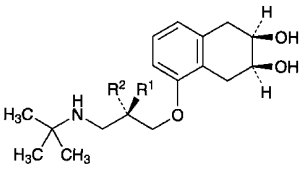
M_S: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside 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 acetonitrile, water and acetic acid (100) (550:450:1).
Flow rate: Adjust the flow rate so that the retention time of nabumetone 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, nabumetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nadolol

ナドロール



and enantiomer

$C_{17}H_{27}NO_4$: 309.40

R^1 = OH, R^2 = H

(2*RS*,3*SR*)-5-{(2*SR*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol

R^1 = H, R^2 = OH

(2*RS*,3*SR*)-5-{(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol

[*42200-33-9*]

Nadolol, when dried, contains not less than 98.0% of $C_{17}H_{27}NO_4$.

Description Nadolol occurs as a white to yellow-brownish white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in water and in chloroform.

A solution of Nadolol in methanol (1 in 100) shows no optical rotation.

Melting point: about 137°C.

Identification **(1)** Determine the absorption spectrum of a solution of Nadolol in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1585 cm^{-1}, 1460 cm^{-1}, 1092 cm^{-1}, 935 cm^{-1} and 770 cm^{-1}.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nadolol 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 0.5 g of Nadolol in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 100 μL each of the sample solution and a mixture of methanol and chloroform (1:1) as a control solution with 25 mm each of width at an interval of about 10 mm on the starting line of a plate 0.25 mm in thickness of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform and diluted ammonia TS (1 in 3) (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm), and confirm the positions of the principal spot and the spots other than the principal spot from the sample solution. Scratch and collect the silica gel of the positions of the plate corresponding to the principal spot and the spots other than the principal spot. To the silica gel collected from the principal spot add exactly 30 mL of ethanol (95), and to the silica gel from the spots other than the principal spot add exactly 10 mL of ethanol (95). After shaking them for 60 minutes, centrifuge, and determine the absorbances of these supernatant liquids at 278 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Separately, proceed in the same manner with each position of the silica gel from the control solution corresponding to the principal spot and the spots other than the principal spot of the sample solution, and perform a blank determination to make correction. Amount of the related substances calculated by the following equation is not more than 2.0%.

$$\text{Amount (\%) of related substances} = A_b/(A_b + 3A_a) \times 100$$

A_a: Corrected absorbance of the principle spot

A_b: Corrected absorbance of the spots other than the principle spot

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer ratio Prepare a paste with 0.01 g of Nadolol as directed in the paste method under Infrared Spectrophotometry <*2.25*> so that its transmittance at an absorption band at a wave number of about 1585 cm^{-1} is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm^{-1} and 1100 cm^{-1}. Determine the absorbances, A_{1265} and A_{1250}, from the transmittances, T_{1265} and T_{1250}, at wave numbers of about 1265 cm^{-1} (racemic substance A) and 1250 cm^{-1} (racemic substance B), respectively: the ratio A_{1265}/A_{1250} is between 0.72 and 1.08.

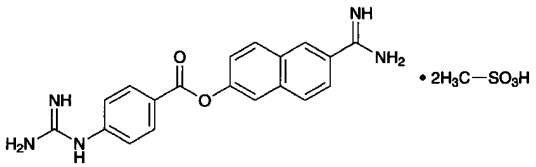
Assay Weigh accurately about 0.28 g of Nadolol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green-blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.94 mg of $C_{17}H_{27}NO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Nafamostat Mesilate

ナファモスタットメシル酸塩



$C_{19}H_{17}N_5O_2.2CH_4O_3S$: 539.58
6-Amidinonaphthalen-2-yl 4-guanidinobenzoate bis(methanesulfonate)
[*82956-11-4*]

Nafamostat Mesilate, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{17}N_5O_2.2CH_4O_3S$.

Description Nafamostat Mesilate occurs as a white crystalline powder.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 262°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nafamostat Mesilate in 0.01 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nafamostat Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A 0.1-g portion of Nafamostat Mesilate responds to the Qualitative Tests <*1.09*> (1) for mesilate.

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is between 4.7 and 5.7.

Purity (1) Clarity and color of solution—A solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Nafamostat 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).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Nafamostat Mesilate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mobile phase to make exactly 100 mL. Then pipet 5 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than nafamostat obtained from the sample solution is not larger than 1/5 times the peak area of nafamostat from the standard solution. Furthermore, the total area of the peaks other than nafamostat is not larger than the peak area of nafamostat from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: Dissolve 6.07 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (3 in 500). To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nafamostat is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of nafamostat, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of nafamostat obtained from 10 μL of this solution is equivalent to 1.1 to 1.9% of that from 10 μL of the standard solution.

System performance: Dissolve 0.1 g of nafamostat mesilate in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 100 mL. To 5 mL of this solution add 5 mL of a solution of 6-amidino-2-naphthol methanesulfonate in the mobile phase (1 in 20,000). When the procedure is run with 10 μL of this solution under the above operating conditions, 6-amidino-2-naphthol and nafamostat 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nafamostat is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

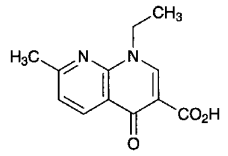
Assay Weigh accurately about 0.25 g of Nafamostat Mesilate, previously dried, dissolve in 4 mL of formic acid, add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.98 mg of $C_{19}H_{17}N_5O_2.2CH_4O_3S$

Containers and storage Containers—Tight containers.

Nalidixic Acid

ナリジクス酸



$C_{12}H_{12}N_2O_3$: 232.24
1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid
[*389-08-2*]

Nalidixic Acid, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$.

Description Nalidixic Acid occurs as white to light yellow crystals or crystalline powder.

It is sparingly soluble in *N*,*N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Nalidixic Acid in 0.01 mol/L sodium hydroxide TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nalidixic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 225 – 231°C.

Purity **(1)** Chloride <*1.03*>—To 2.0 g of Nalidixic Acid add 50 mL of water, warm at 70°C for 5 minutes, cool quickly, 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 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Nalidixic 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).

(3) Related substances—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. To 5 mL of this solution, add water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nalidixic acid with the sample solution is not larger than the peak area of nalidixic acid with the standard solution, and the total area of the peaks other than the peak of nalidixic acid is not larger than 2.5 times the peak area of nalidixic acid with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in this order with the resolution between these peaks being not less than 13.

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

Loss on drying <*2.41*> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

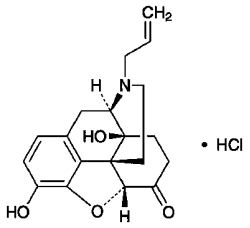
Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of *N*,*N*-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS
= 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Tight containers.

Naloxone Hydrochloride

ナロキソン塩酸塩



$C_{19}H_{21}NO_4$.HCl: 363.84
(5*R*,14*S*)-17-Allyl-4,5-epoxy-3,14-dihydroxymorphinan-6-one monohydrochloride
[*357-08-4*]

Naloxone Hydrochloride contains not less than 98.5% of $C_{19}H_{21}NO_4$.HCl, calculated on the dried basis.

Description Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic.

It is gradually colored by light.

Identification **(1)** Determine the absorption spectrum of a solution of Naloxone Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Naloxone Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: -170 – $-181°$ (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

Purity Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.1 g).

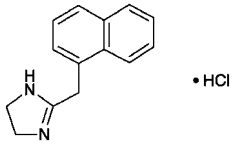
Assay Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.38 mg of $C_{19}H_{21}NO_4$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naphazoline Hydrochloride

ナファゾリン塩酸塩



$C_{14}H_{14}N_2$.HCl: 246.74
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole monohydrochloride
[*550-99-2*]

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of $C_{14}H_{14}N_2$.HCl.

Description Naphazoline Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, soluble in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 255 – 260°C (with decomposition).

Identification **(1)** To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts ⟨*2.60*⟩ between 117°C and 120°C.

(3) Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 0.10 g of Naphazoline Hydrochloride

in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Naphazoline 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).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

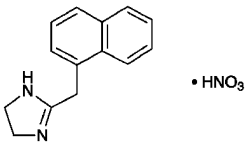
Assay Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.67 mg of $C_{14}H_{14}N_2.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naphazoline Nitrate

ナファゾリン硝酸塩



$C_{14}H_{14}N_2.HNO_3$: 273.29
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole mononitrate
[*5144-52-5*]

Naphazoline Nitrate, when dried, contains not less than 98.5% of $C_{14}H_{14}N_2.HNO_3$.

Description Naphazoline Nitrate occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in water, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 20 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C for 1 hour: the residue so obtained melts <*2.60*> between 117°C and 120°C.

(3) A solution of Naphazoline Nitrate (1 in 20) responds to the Qualitative Tests <*1.09*> for nitrate.

pH <*2.54*> Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Melting point <*2.60*> 167 – 170°C.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Naphazoline Nitrate 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 <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Naphazoline Nitrate, previously dried, dissolve in 10 mL of acetic acid (100) and 40 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (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
= 27.33 mg of $C_{14}H_{14}N_2.HNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naphazoline and Chlorpheniramine Solution

ナファゾリン･クロルフェニラミン液

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% and not more than 0.055 w/v% of naphazoline nitrate ($C_{14}H_{14}N_2.HNO_3$: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86).

Method of preparation

Naphazoline Nitrate	0.5 g
Chlorpheniramine Maleate	1 g
Chlorobutanol	2 g
Glycerin	50 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve, and mix the above ingredients.

Description Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

Identification **(1)** To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

(2) Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).

(3) To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distil off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate RS in 10 mL and 5 mL of methanol, respectively, and use these solutions as standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same *R*f values as the spots from standard solutions (1) and (2). Spray evenly Dragendorff's TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

Assay Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105°C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate RS, dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the standard solution.

Amount (mg) of naphazoline nitrate ($C_{14}H_{14}N_2.HNO_3$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25$

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25$

M_{Sa}: Amount (mg) of naphazoline nitrate for assay
M_{Sb}: Amount (mg) of Chlorpheniramine Maleate RS

Internal standard solution—A solution of ethenzamide in methanol (1 in 1000).

Operating conditions—

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

Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).

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

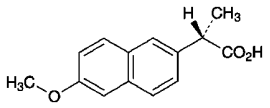
Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline and chlorpheniramine in this order.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Naproxen

ナプロキセン



$C_{14}H_{14}O_3$: 230.26
(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid
[*22204-53-1*]

Naproxen, when dried, contains not less than 98.5% of $C_{14}H_{14}O_3$.

Description Naproxen occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

(2) To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxylamine perchlorate-dehydrated ethanol TS and 1 mL of *N*,*N*′-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a red-purple color develops.

(3) Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Naproxen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: +63.0 – +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 154 – 158°C.

Purity **(1)** Clarity of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the

test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 400 nm is not more than 0.070.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Naproxen 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 <*1.11*>—Prepare the test solution with 2.0 g of Naproxen according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of chloroform and ethanol (99.5) (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of chloroform and ethanol (99.5) (1:1) 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 <*2.03*>. Spot 10 μL each of the sample 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, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) 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 and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming if necessary, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

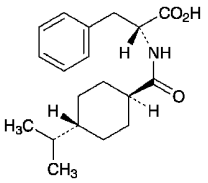
Each mL of 0.1 mol/L sodium hydroxide VS
= 23.03 mg of $C_{14}H_{14}O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nateglinide

ナテグリニド



$C_{19}H_{27}NO_3$: 317.42

N-[*trans*-4-(1-Methylethyl)cyclohexanecarbonyl]-D-phenylalanine [*105816-04-4*]

Nateglinide, when dried, contains not less than 98.0% and not more than 102.0% of $C_{19}H_{27}NO_3$.

Description Nateglinide occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), sparingly soluble in acetonitrile, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Nateglinide in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nateglinide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nateglinide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Nateglinide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the reference standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -36.5 – $-40.0°$ (after drying 0.2 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nateglinide 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 0.25 g of Nateglinide in 20 mL of acetonitrile. To 4 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nateglinide from the sample solution is not larger than the peak area of nateglinide 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.

Time span of measurement: About 4 times as long as the retention time of nateglinide, beginning after the solvent peak.

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 the symmetry factor of the peak of nateglinide are not less than 6000 and not more than 1.2, respectively.

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

(3) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.2% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Nateglinide, previously dried, and dissolve in acetonitrile to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried, and dissolve in acetonitrile to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nateglinide to that of the internal standard.

$$\text{Amount (mg) of } C_{19}H_{27}NO_3 = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Nateglinide RS

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 500).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Adjust 0.05 mol/L sodium dihydrogen phosphate TS to pH 2.5 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of nateglinide 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 nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nateglinide Tablets

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Nateglinide Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of nateglinide ($C_{19}H_{27}NO_3$: 317.42).

Method of preparation Prepare as directed under Tablets, with Nateglinide.

Identification To an amount of powdered Nateglinide Tablets, equivalent to 20 mg of Nateglinide according to the labeled amount, add 20 mL of methanol, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 246 nm and 250 nm, between 251 nm and 255 nm, between 257 nm and 261 nm and between 262 nm and 266 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nateglinide Tablets add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablet, and disperse to fine particles with the aid of ultrasonic waves. Add exactly $3V/50$ mL of the internal standard solution, add $3V/5$ mL of acetonitrile, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 0.6 mg of nateglinide ($C_{19}H_{27}NO_3$). Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, and discard the first 5 mL of the filtrate. To 8 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 10 mL. Pipet 6 mL of this solution, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. To 8 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and calculate the ratios, Q_T and Q_S, of the peak area of nateglinide to that of the internal standard.

$$\text{Amount (mg) of nateglinide } (C_{19}H_{27}NO_3) = M_S \times Q_T/Q_S \times 3V/250$$

M_S: Amount (mg) of Nateglinide RS

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 250).

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 under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of a 30-mg tablet and that in 30 minutes of a 90-mg tablet of Nateglinide Tablets is not less than 75%, respectively.

Start the test with 1 tablet of Nateglinide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 medium to make exactly V' mL so that each mL contains about 33 μg of nateglinide ($C_{19}H_{27}NO_3$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of nateglinide of both solutions.

Dissolution rate (%) with respect to the labeled amount of nateglinide ($C_{19}H_{27}NO_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Nateglinide RS
C: Labeled amount (mg) of nateglinide ($C_{19}H_{27}NO_3$) 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 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nateglinide are not less than 8000 and not more than 1.5, respectively.

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

Assay To 20 Nateglinide Tablets add $V/5$ mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablets, and disperse to fine particles with the aid of ultrasonic waves. Then, add $V/2$ mL of acetonitrile and exactly $V/10$ mL of the internal standard solution, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 6 mg of nateglinide ($C_{19}H_{27}NO_3$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, to 4 mL of the subsequent filtrate add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Nateglinide RS, previously dried at 105°C for 2 hours, add exactly 1 mL of the internal standard solution, and add acetonitrile to make 10 mL. To 4 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nateglinide to that of the internal standard.

Amount (mg) of nateglinide ($C_{19}H_{27}NO_3$) in 1 tablet
$= M_S \times Q_T/Q_S \times V/200$

M_S: Amount (mg) of Nateglinide RS

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (3 in 125).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Adjust to pH 2.5 of 0.05 mol/L sodium dihydrogen phosphate TS with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.

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

System suitability—

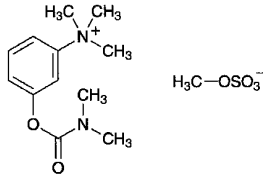
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Neostigmine Methylsulfate

ネオスチグミンメチル硫酸塩



$C_{13}H_{22}N_2O_6S$: 334.39
3-(Dimethylcarbamoyloxy)-*N*,*N*,*N*-trimethylanilinium methyl sulfate
[*51-60-5*]

Neostigmine Methylsulfate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{13}H_{22}N_2O_6S$.

Description Neostigmine Methylsulfate occurs as a white, crystalline powder.

It is very soluble in water, and freely soluble in acetonitrile and in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Neostigmine Methylsulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Neostigmine Methylsulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Neostigmine Methylsulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

Melting point <*2.60*> 145 – 149°C.

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

(2) Sulfate—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

(3) Dimethylaminophenol—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, add 1 mL of sodium hydroxide TS, and while cooling with ice, add 1 mL of diazobenzenesulfonic acid TS: no color develops.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS, previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of neostigmine in each solution.

$$\text{Amount (mg) of } C_{13}H_{22}N_2O_6S = M_S \times A_T/A_S$$

M_S: Amount (mg) of Neostigmine Methylsulfate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 259 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 3.12 g of sodium dihydrogenphosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of neostigmine is about 9 minutes.

System suitability—

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, dimethylaminophenol and neostigmine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine methylsulfate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Neostigmine Methylsulfate Injection

ネオスチグミンメチル硫酸塩注射液

Neostigmine Methylsulfate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$: 334.39).

Method of preparation Prepare as directed under Injections, with Neostigmine Methylsulfate.

Description Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

Identification Take a volume of Neostigmine Methylsulfate Injection equivalent to 5 mg of neostigmine methylsulfate according to the labeled amount, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectro-

photometry <*2.24*>: it exhibits a maximum between 257 nm and 261 nm.

Bacterial endotoxins <*4.01*> Less than 5 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Use Neostigmine Methylsulfate Injection as the sample solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate RS, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.

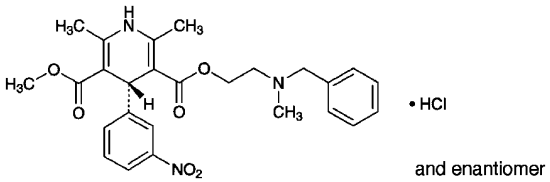
Amount (mg) of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Neostigmine Methylsulfate RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Nicardipine Hydrochloride

ニカルジピン塩酸塩



$C_{26}H_{29}N_3O_6$.HCl: 515.99
2-[Benzyl(methyl)amino]ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride
[*54527-84-3*]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of $C_{26}H_{29}N_3O_6$.HCl.

Description Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

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

It is gradually affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Nicardipine Hydrochloride in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.02 g of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 167 – 171°C.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nicardipine 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).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, then take exactly 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than the peak of nicardipine is not larger than 2 times the peak area of nicardipine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 30°C.

Mobile phase: A mixture of a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 6 minutes.

Time span of measurement: About 4 times as long as the retention time of nicardipine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, nicardipine and nifedipine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 3%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ 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
= 51.60 mg of $C_{26}H_{29}N_3O_6$.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicardipine Hydrochloride Injection

ニカルジピン塩酸塩注射液

Nicardipine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl: 515.99).

Method of preparation Prepare as directed under Injections, with Nicardipine Hydrochloride.

Description Nicardipine Hydrochloride Injection occurs as a clear pale yellow liquid.

It is gradually changed by light.

Identification To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

pH ⟨*2.54*⟩ 3.0 – 4.5

Purity Related substances—Conduct the procedure without exposure to day-light using light-resistant vessels. To a volume of Nicardipine Hydrochloride Injection, equivalent to 5 mg of Nicardipine Hydrochloride according to the labeled amount, add the mobile phase to make 10 mL, and use this solution as the sample solution. To exactly 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of these solutions by the automatic integration method: the areas of the peaks other than nicardipine from the sample solution are not larger than the peak area of nicardipine from the standard solution, and the total of the areas of the peaks other than nicardipine is not larger than 2 times of the peak area of nicardipine 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.

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

System suitability—

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 nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

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

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 8.33 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Conduct the procedure without exposure to daylight using light-resistant vessels. To an exact volume of Nicardipine Hydrochloride Injection, equivalent to about 2 mg of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nicardipine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nicardipine to that of the internal standard.

Amount (mg) of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl)
$= M_S \times Q_T/Q_S \times 1/25$

M_S: Amount (mg) of nicardipine hydrochloride for assay

Internal standard solution—A solution of di-*n*-butyl phthalate in methanol (1 in 625).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL. To 320 mL of this solution add 680 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicardipine 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, nicardipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

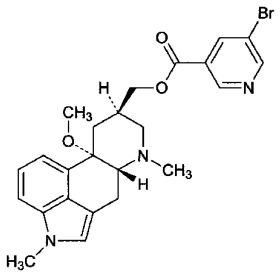
System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Nicergoline

ニセルゴリン



$C_{24}H_{26}BrN_3O_3$: 484.39

[(8*R*,10*S*)-10-Methoxy-1,6-dimethylergolin-8-yl]methyl 5-bromopyridine-3-carboxylate

[*27848-84-6*]

Nicergoline, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{26}BrN_3O_3$.

Description Nicergoline occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Nicergoline in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicergoline as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +5.2 – +6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nicergoline 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 Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than the peak of nicergoline is not larger than 7.5 times the peak area of nicergoline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside 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: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 4.0%.

Loss on drying <*2.41*> Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.22 mg of $C_{24}H_{26}BrN_3O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

Method of preparation Prepare as directed under Granules or Powders, with Nicergoline.

Identification Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline according to the labeled amount, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them 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.

Time span of measurement: About 2 times as long as the retention time of nicergoline after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage unit ⟨*6.02*⟩ The Nicergoline Powder in single-unit container meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Nicergoline Powder is not less than 80%.

Start the test with an accurately weighed amount of Nicergoline Powder, equivalent to about 5 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$) according to the labeled amount, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a laminated polyester fiber. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, A_{T1} and A_{S1}, and at 250 nm, A_{T2} and A_{S2}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$)
$= M_S/M_T \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times 1/C \times 9$

M_S: Amount (mg) of nicergoline for assay
M_T: Amount (g) of sample
C: Labeled amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$) in 1 g

Assay Weigh accurately a quantity of Nicergoline Powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of nicergoline.

Amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of nicergoline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside 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

40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 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 nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

Method of preparation Prepare as directed under Tablets, with Nicergoline.

Identification Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline according to the labeled amount, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a 0.45-μm pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them 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.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet exactly 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_{T1} and A_{S1}, and at 340 nm, A_{T2} and A_{S2}, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times 1/2$$

M_S: Amount (mg) of nicergoline for assay

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of nicergoline.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of nicergoline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—

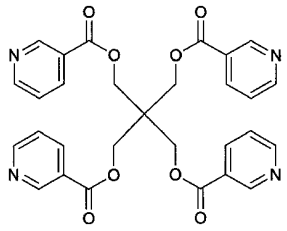
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Niceritrol

ニセリトロール



$C_{29}H_{24}N_4O_8$: 556.52
Pentaerythritol tetranicotinate
[*5868-05-3*]

Niceritrol, when dried, contains not less than 99.0% of $C_{29}H_{24}N_4O_8$.

Description Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, soluble in *N*,*N*-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Niceritrol in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Niceritrol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 162 – 165°C.

Purity **(1)** Chloride <*1.03*>—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. 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 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Niceritrol 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 <*1.11*>—Prepare the test solution with 1.0 g of Niceritrol according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Pyridine—Dissolve 0.5 g of Niceritrol in *N*,*N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine each peak area of pyridine in both solutions: the peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 2 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical steps of the peak of pyridine is not less than 1500 steps.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pyridine is not more than 3.0%.

(5) Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separator, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate <*2.50*> with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

$$\text{Each mL of 0.01 mol/L sodium hydroxide VS} = 1.231 \text{ mg of } C_6H_5NO_2$$

(6) Related substances—Dissolve 0.10 g of Niceritrol in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet exactly 2 mL of this solution, add chloroform 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 <*2.03*>. Spot 10 μL each of the sample 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 and ethanol (95) (4:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

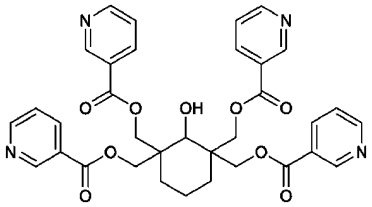
Assay Weigh accurately about 1 g of Niceritrol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate ⟨*2.50*⟩ immediately the excess sodium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 69.57 mg of $C_{29}H_{24}N_4O_8$

Containers and storage Containers—Well-closed containers.

Nicomol

ニコモール



$C_{34}H_{32}N_4O_9$: 640.64
(2-Hydroxycyclohexane-1,1,3,3-tetrayl)tetramethyl tetranicotinate
[*27959-26-8*]

Nicomol, when dried, contains not less than 98.0% of $C_{34}H_{32}N_4O_9$.

Description Nicomol occurs as a white, crystalline powder. It is odorless and tasteless.

It is soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification **(1)** Mix 0.01 g of Nicomol with 0.02 g of 1-chloro-2,4-dinitrobenzene, add 2 mL of dilute ethanol, heat in a water bath for 5 minutes, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Dissolve 0.1 g of Nicomol in 5 mL of dilute hydrochloric acid, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Nicomol in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nicomol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 181 – 185°C.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Nicomol in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Nicomol add 50 mL of freshly boiled and cooled water, shake for 5 minutes, filter, and to 25 mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(3) Chloride ⟨*1.03*⟩—Dissolve 0.6 g of Nicomol 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 as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nicomol 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Nicomol according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Nicomol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 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 these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, and boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 10 minutes. After cooling, titrate ⟨*2.50*⟩ immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 80.08 mg of $C_{34}H_{32}N_4O_9$

Containers and storage Containers—Tight containers.

Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$: 640.64).

Method of preparation Prepare as directed under Tablets, with Nicomol.

Identification To a portion of powdered Nicomol Tablets, equivalent to 0.5 g of Nicomol according to the labeled amount, add 20 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Nicomol Tablets is not less than 75%.

Start the test with 1 tablet of Nicomol Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 18 μg of nicomol ($C_{34}H_{32}N_4O_9$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL, then pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of nicomol for assay
C: Labeled amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$) in 1 tablet

Assay Weigh accurately not less than 20 Nicomol Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of nicomol ($C_{34}H_{32}N_4O_9$), add 100 mL of 1 mol/L hydrochloric acid TS, shake well, add water to make exactly 500 mL, and filter. Discard the first 50 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 50 mL of 1 mol/L hydrochloric acid TS and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in 50 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add 20 mL of 1 mol/L hydrochloric acid TS and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

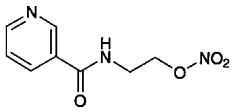
Amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$)
$= M_S \times A_T/A_S \times 25/2$

M_S: Amount (mg) of nicomol for assay

Containers and storage Containers—Tight containers.

Nicorandil

ニコランジル



$C_8H_9N_3O_4$: 211.17
N-[2-(Nitrooxy)ethyl]pyridine-3-carboxamide
[*65141-46-0*]

Nicorandil contains not less than 98.5% and not more than 101.0% of $C_8H_9N_3O_4$, calculated on the anhydrous basis.

Description Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Nicorandil (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicorandil as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Sulfate ⟨*1.14*⟩—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, 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, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Nicorandil 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 20 mg of Nicorandil in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of *N*-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and

the sum area of the peaks other than nicorandil and *N*-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25% of the total peak area.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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, triethylamine and trifluoroacetic acid (982:10:5:3).

Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.

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

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 μL of this solution is equivalent to 2 to 8% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of *N*-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the sample solution. When the procedure is run with this solution under the above operating conditions, *N*-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil 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 of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5%.

Water ⟨*2.48*⟩ Not more than 0.1% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

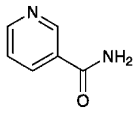
Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_8H_9N_3O_4$

Containers and storage Containers—Tight containers.

Storage—At a temperature between 2°C and 8°C.

Nicotinamide

ニコチン酸アミド



$C_6H_6N_2O$: 122.12
Pyridine-3-carboxamide
[*98-92-0*]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of $C_6H_6N_2O$.

Description Nicotinamide occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Identification **(1)** Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a red color is produced.

(2) To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3) Dissolve 0.02 g of Nicotinamide in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

Melting point ⟨*2.60*⟩ 128 – 131°C

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

(2) Chloride ⟨*1.03*⟩—Take 0.5 g of Nicotinamide, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nicotinamide 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).

(5) Readily carbonizable substances ⟨*1.15*⟩—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Nicotinamide

and Nicotinamide RS, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make exactly 100 mL. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nicotinamide to that of the internal standard.

Amount (g) of nicotinamide ($C_6H_6N_2O$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of dried Nicotinamide RS

Internal standard solution—A solution of nicotinic acid (1 in 25,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 7 minutes.

System suitability—

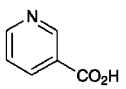
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Nicotinic Acid

ニコチン酸



$C_6H_5NO_2$: 123.11
Pyridine-3-carboxylic acid
[*59-67-6*]

Nicotinic Acid, when dried, contains not less than 99.5% of $C_6H_5NO_2$.

Description Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

Identification **(1)** Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

(2) Dissolve 0.02 g of Nicotinic Acid in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH <2.54> Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

Melting point <2.60> 234 – 238°C

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of Nicotinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 1.0 g of Nicotinic Acid in 3 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 and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

(4) Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Nicotinic 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).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.31 mg of $C_6H_5NO_2$

Containers and storage Containers—Well-closed containers.

Nicotinic Acid Injection

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Nicotinic Acid Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 110.0% of the labeled amount of nicotinic acid ($C_6H_5NO_2$: 123.11).

Method of preparation Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

Description Nicotinic Acid Injection is a clear, colorless liquid.

pH: 5.0 – 7.0

Identification **(1)** To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid according to the labeled amount, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt ⟨*2.60*⟩ between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

(2) Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, A_1 and A_2, at each wavelength of maximum and minimum absorption, respectively: the ratio A_2/A_1 is between 0.35 and 0.39.

Bacterial endotoxins ⟨*4.01*⟩ Less than 3.0 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid ($C_6H_5NO_2$), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nicotinic acid to that of the internal standard.

$$\text{Amount (mg) of nicotinic acid } (C_6H_5NO_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Nicotinic Acid RS

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: Dissolve 1.1 g of sodium 1-octane sulfonate in a mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and methanol (4:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of caffeine 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, nicotinic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Nifedipine

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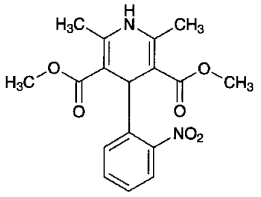

$C_{17}H_{18}N_2O_6$: 346.33

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

[*21829-25-4*]

Nifedipine contains not less than 98.0% and not more than 102.0% of $C_{17}H_{18}N_2O_6$, calculated on the dried basis.

Description Nifedipine occurs as a yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water.

It is affected by light.

Identification **(1)** Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g

of zinc powder. Allow to stand for 5 minutes, and filter. Perform the test with the filtrate as directed under Qualitative Tests <*1.09*> for primary aromatic amines: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Nifedipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Nifedipine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 172 – 175°C.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

(2) Chloride <*1.03*>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent 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%).

(3) Sulfate <*1.14*>—To 4 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 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Nifedipine 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 <*1.11*>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).

(6) Basic substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

(7) Dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay The procedure should be performed under protection from direct sunlight in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 350 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{17}H_{18}N_2O_6 = A/142.3 \times 40{,}000$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Nilvadipine

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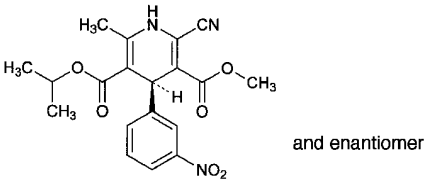

and enantiomer

$C_{19}H_{19}N_3O_6$: 385.37
3-Methyl 5-(1-methylethyl) (4*RS*)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*75530-68-6*]

Nilvadipine contains not less than 98.0% and not more than 102.0% of $C_{19}H_{19}N_3O_6$.

Description Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Nilvadipine in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nilvadipine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nilvadipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Nilvadipine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 167 – 171°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Nilvadipine 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 Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside 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 phosphate buffer solution, pH 7.4, methanol and acetonitrile (32:27:18).

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

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 and the symmetry factor of the peak of nilvadipine is not less than 3300 and not more than 1.3, respectively.

System repeatability: Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Nilvadipine and Nilvadipine RS, dissolve in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 20 mL of the internal standard solution, 20 mL of water and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under the Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nilvadipine to that of the internal standard.

$$\text{Amount (mg) of } C_{19}H_{19}N_3O_6 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in methanol (1 in 200).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nilvadipine Tablets

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Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine ($C_{19}H_{19}N_3O_6$: 385.37).

Method of preparation Prepare as directed under Tablets, with Nilvadipine.

Identification To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of Nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine ($C_{19}H_{19}N_3O_6$), add

exactly V mL of the internal standard solution, and disperse the particles with the aid of ultrasonic waves. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of nilvadipine } (C_{19}H_{19}N_3O_6) = M_S \times Q_T/Q_S \times V/100$$

M_S: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Nilvadipine Tablets is not less than 85%.

Start the test with 1 tablet of Nilvadipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine RS, equivalent to 10 times the labeled amount of Nilvadipine Tablets, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of water, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of nilvadipine.

$$\text{Dissolution rate (\%) with respect to the labeled amount of nilvadipine } (C_{19}H_{19}N_3O_6) = M_S \times A_T/A_S \times 1/C \times 9$$

M_S: Amount (mg) of Nilvadipine RS
C: Labeled amount (mg) of nilvadipine ($C_{19}H_{19}N_3O_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4 mm in inside 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 phosphate buffer solution, pH 7.4, methanol and acetonitrile (7:7:6).

Flow rate: Adjust the flow rate so that the retention time of nilvadipine 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 and the symmetry factor of the peak of nilvadipine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Assay Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine ($C_{19}H_{19}N_3O_6$), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nilvadipine to that of the internal standard.

$$\text{Amount (mg) of nilvadipine } (C_{19}H_{19}N_3O_6) = M_S \times Q_T/Q_S \times 1/4$$

M_S: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydoxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—

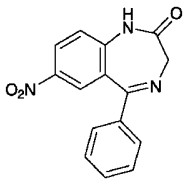
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nitrazepam

ニトラゼパム



$C_{15}H_{11}N_3O_3$: 281.27
7-Nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*146-22-5*]

Nitrazepam, when dried, contains not less than 99.0% of $C_{15}H_{11}N_3O_3$.

Description Nitrazepam occurs as white to yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

Identification **(1)** To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

(2) To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

(4) Determine the absorption spectrum of a solution of Nitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Nitrazepam 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 <*1.11*>—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

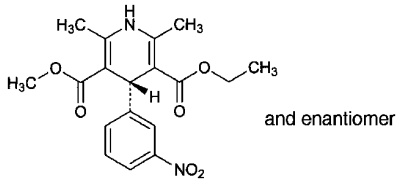
Assay Weigh accurately about 0.4 g of Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). Titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.13 mg of $C_{15}H_{11}N_3O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Nitrendipine

ニトレンジピン



$C_{18}H_{20}N_2O_6$: 360.36
3-Ethyl 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*39562-70-4*]

Nitrendipine, when dried, contains not less than 98.5% and not more than 101.0% of $C_{18}H_{20}N_2O_6$.

Description Nitrendipine occurs as a yellow crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nitrendipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 157 – 161°C.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of

Nitrendipine 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—Conduct this procedure rapidly using light-resistant vessels. Dissolve 40 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of related substances by the following equation: the amount of a related substance, having the relative retention time of about 0.8 with respect to nitrendipine, is not more than 1.0%, a related substance, having the relative retention time of about 1.3, is not more than 0.25%, and other related substances are not more than 0.2%, respectively. The total amount of the substances other than nitrendipine is not more than 2.0%.

$$\text{Amount (\%) of related substance} = A_T/A_S$$

A_T: Each peak area other than nitrendipine obtained from the sample solution

A_S: Peak area of nitrendipine obtained from the standard solution

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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 water, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nitrendipine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of nitrendipine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: Dissolve 10 mg of Nitrendipine and 3 mg of propyl parahydroxybenzoate in 5 mL of acetonitrile, and add the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nitrendipine, previously dried, dissolve in 60 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water, and titrate <2.50> with 0.1 mol/L serium (IV) tetraammonium sulfate VS until the red-orange color of the solution vanishes (indicator: 3 drops of 1,10-phenanthroline TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L serium (IV) tetraammonium sulfate VS
= 18.02 mg of $C_{18}H_{20}N_2O_6$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Nitrendipine Tablets

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Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$: 360.36).

Method of preparation Prepare as directed under Tablets, with Nitrendipine.

Identification Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine according to the labeled amount, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

Uniformity of dosage units <6.02> 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 Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrated, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of nitrendipine } (C_{18}H_{20}N_2O_6) = M_S \times Q_T/Q_S \times 1/V \times 1/5$$

M_S: Amount (mg) of nitrendipine for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium containing 3 g of polysorbate 80 in 5 L of water for 5-mg tablet and the dissolution medium containing 3 g of polysorbate 80 in 2000 mL of

water for 10-mg tablet, the dissolution rate in 45 minutes of Nitrendipine Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Nitrendipine Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of nitrendipine ($C_{18}H_{20}N_2O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of nitrendipine.

Dissolution rate (%) with respect to the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of nitrendipine for assay
C: Labeled amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 356 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust the flow rate so that the retention time of nitrendipine 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 number of theoretical plates and the symmetry factor of the peak of nitrendipine are not less than 5000 and not more than 2.0, respectively.

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

Assay Conduct this procedure using light-resistant vessels. To 20 tablets of Nitrendipine Tablets add 150 mL of diluted acetonitrile (4 in 5), stir until the tablets completely disintegrate, and stir for further 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 200 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 2 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 10 mL of the internal standard solution and diluted acetonitrile (4 in 5) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nitrendipine for assay, previously dried at 105°C for 2 hours, and dissolve in diluted acetonitrile (4:5) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution and diluted acetonitrile (4:5) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nitrendipine to that of the internal standard.

Amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$)
$= M_S \times Q_T/Q_S \times 1/50$

M_S: Amount (mg) of nitrendipine for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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 water, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust the flow rate so that the retention time of nitrendipine 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, the internal standard and nitrendipine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nitrogen

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N_2: 28.01

Nitrogen is the nitrogen produced by the air liquefaction separation method.

It contains not less than 99.5 vol% of N_2.

Description Nitrogen is a colorless gas at room temperature and under atmospheric pressure, and is odorless.

1 mL of Nitrogen dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Nitrogen at 0°C and at a pressure of 101.3 kPa weighs 1.251 g.

Identification Introduce 1 mL each of Nitrogen and nitrogen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless

steel tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the following conditions: the principal peak obtained form Nitrogen has the same retention time with the peak from nitrogen.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

Purity Oxygen—The peak area of oxygen obtained from Nitrogen in the Assay is not larger than 1/2 times that obtained from the standard gas mixture.

Assay Introduce 1.0 mL of Nitrogen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless steel tube. Perform the test with this gas as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak area A_T of oxygen. Separately, introduce 1.0 mL of oxygen into the gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard gas mixture. Proceed with 1.0 mL of this mixture in the same manner under Nitrogen, and measure the peak area A_S of oxygen.

$$\text{Amount (vol\%) of } N_2 = 100 - A_T/A_S$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography (250 to 355 μm in particle diameter; 0.5 nm in pore size).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

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

System suitability—

System performance: Introduce 1.0 mL of oxygen into the gas mixer, add Nitrogen to make 100 mL, and mix thoroughly. When the procedure is run with 1.0 mL of this mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard gas mixture under the above conditions, the relative standard deviation of the peak area of oxygen is not more than 2.0%.

Containers and storage Containers—Pressure-resistant cylinders.

Storage—Not exceeding 40°C.

Nitroglycerin Tablets

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Nitroglycerin Tablets contain not less than 80.0% and not more than 120.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$: 227.09).

Method of preparation Prepare as directed under Tablets, with nitroglycerin.

Identification (1) Weigh a quantity of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

(2) Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

Purity Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, to a separator, add 40 mL of isopropylether and 40 mL of water, shake for 10 minutes, and allow the layers to separate. Collect the aqueous layer, add 40 mL of isopropylether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 30 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, continue the procedure in the same manner as the sample solution, and use the solution so obtained as the standard solution. Transfer 20 mL each of the sample solution and the standard solution to Nessler tubes, respectively, shake well with 30 mL of water and 0.06 g of Griess-Romijin's nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than the standard solution.

Uniformity fo dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, and add exactly V mL of acetic acid (100) to provide a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

Amount (mg) of nitroglycerin ($C_3H_5N_3O_9$)
$= M_S \times A_T/A_S \times V/2000 \times 0.749$

M_S: Amount (mg) of potassium nitrate

Calculate the average content from the contents of 10 tablets: it meets the requirements of the test when each content deviates from the average content by not more than 25%. When there is 1 tablet showing a deviation exceeding 25% and not exceeding 30%, determine the content of an additional 20 tablets in the same manner. Calculate the 30 deviations from the new average of all 30 tablets: it meets the requirements of the test when 1 tablet may deviate from the average content by between 25% and 30%, but no tablet deviates by more than 30%.

Disintegration <*6.09*> It meets the requirement, provided that the time limit of the test is 2 minutes, and the use of the disks is omitted.

Assay Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin ($C_3H_5N_3O_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

Amount (mg) of nitroglycerin ($C_3H_5N_3O_9$)
$= M_S \times A_T/A_S \times 1/20 \times 0.749$

M_S: Amount (mg) of potassium nitrate

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and not exceeding 20°C.

Nitrous Oxide

亜酸化窒素

N_2O: 44.01

Nitrous Oxide contains not less than 97.0 vol% of N_2O.

Description Nitrous Oxide is a colorless gas at room temperature and at atmospheric pressure, and is odorless.

1 mL of Nitrous Oxide dissolves in 1.5 mL of water and in 0.4 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa. It is soluble in diethyl ether and in fatty oils.

1000 mL of Nitrous Oxide at 0°C and at a pressure of 101.3 kPa weighs about 1.96 g.

Identification **(1)** A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2) Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <*2.02*> according to the conditions of the Assay: the retention time of the main peak from Nitrous Oxide coincides with that of nitrous oxide.

Purity Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and correct the volume at 20°C and at a pressure of 101.3 kPa.

(1) Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 100 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3) Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5) Chloride <*1.03*>—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6) Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatogra-

phy 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 <2.02> under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in pore size).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.

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 selection of column is about 10 cm.

Assay Withdraw Nitrous Oxide as directed in the Purity.

Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area A_S of nitrogen in the same manner.

$$\text{Amount (vol\%) of } N_2O = 100 - 3 \times A_T/A_S$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside 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°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.

System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

Nizatidine

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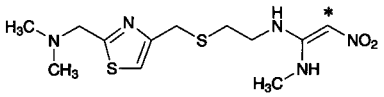


and geometric isomer at C*

$C_{12}H_{21}N_5O_2S_2$: 331.46

(1*EZ*)-*N*-{2-[({2-[(Dimethylamino)methyl]thiazol-4-yl}methyl)sulfanyl]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine

[*76963-41-2*]

Nizatidine, when dried, contains not less than 98.0% and not more than 101.0% of $C_{12}H_{21}N_5O_2S_2$.

Description Nizatidine occurs as a white to pale yellowish white crystalline powder, and has a characteristic odor.

It is soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Nizatidine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nizatidine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nizatidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Nizatidine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 130 – 135°C (after drying).

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Nizatidine according to Method 4, and perform the test using 3 mL of sulfuric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Nizatidine in 10 mL of a mixture of the mobile phase A and mobile phase B (19:6), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of the mobile phase A and mobile phase B (19:6) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than nizatidine peak obtained from the sample solution is not larger than 1/5 times the nizatidine peak area from the standard solution. Furthermore, the total of the areas of peaks other than the nizatidine peak is not larger than the peak area of nizatidine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100).

Mobile phase B: Methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 3	76	24
3 – 20	76 → 50	24 → 50
20 – 45	50	50

Flow rate: 1.0 mL per minute.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of the mobile phase A and mobile phase B (19:6) to make exactly 25 mL. Confirm that the peak area of nizatidine obtained from 50 μL of this solution is equivalent to 15 to 25% of that from 50 μL of the standard solution.

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 nizatidine are not less than 20,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (2 g, 100°C, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 15 mg each of Nizatidine and Nizatidine RS, both previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak area of nizatidine, A_T and A_S, from each solution.

$$\text{Amount (mg) of } C_{12}H_{21}N_5O_2S_2 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Nizatidine RS

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nizatidine 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 the symmetry factor of the peak of nizatidine are not less than 5000 and not more than 1.5, respectively.

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

Containers and storage Containers—Tight containers.

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$: 331.46).

Method of preparation Prepare as directed under Capsules, with Nizatidine.

Identification Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make V mL so that each mL contains about 1.5 mg of nizatidine ($C_{12}H_{21}N_5O_2S_2$). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nizatidine } (C_{12}H_{21}N_5O_2S_2) = M_S \times Q_T/Q_S \times V/10$$

M_S: Amount (mg) of Nizatidine RS

Internal standard solution—A solution of phenol in the mobile phase (1 in 100).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using a sinker, using 900 mL of water as the dissolution medium, the

dissolution rate in 15 minutes of Nizatidine Capsules is not less than 80%.

Start the test with 1 capsule of Nizatidine Capsules, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 10 μg of nizatidine ($C_{12}H_{21}N_5O_2S_2$) according to the labeled amount. Use this solution as the sample solution. Separately, weigh accurately about 25 mg of Nizatidine RS, previously dried at 100°C for 1 hour, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 314 nm.

Dissolution rate (%) with respect to the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Nizatidine RS
C: Labeled amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$) in 1 capsule

Assay Take out the contents of not less than 10 Nizatidine Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of nizatidine ($C_{12}H_{21}N_5O_2S_2$), add exactly 50 mL of the mobile phase, shake vigorously for 10 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Nizatidine RS, previously dried at 100°C for 1 hour, dissolve in 30 mL 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 standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nizatidine to that of the internal standard.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
$= M_S \times Q_T/Q_S \times 10$

M_S: Amount (mg) of Nizatidine RS

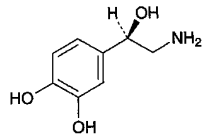
Internal standard solution—A solution of phenol in the mobile phase (1 in 100).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of nizatidine 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 nizatidine 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nizatidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Noradrenaline

Norepinephrine

ノルアドレナリン



and enantiomer

$C_8H_{11}NO_3$: 169.18
4-[(1*RS*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol
[*51-41-2*]

Noradrenaline, when dried, contains not less than 98.0% of *dl*-norepinephrine ($C_8H_{11}NO_3$).

Description Noradrenaline occurs as a white to light brown or slightly reddish brown, crystalline powder.
It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95).
It dissolves in dilute hydrochloric acid.
It gradually changes to brown by air and by light.

Identification **(1)** Determine the absorption spectrum of a solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Noradrenaline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid TS, and add water to make 100 mL: the solution is clear and colorless.
(2) Arterenone—Dissolve 50 mg of Noradrenaline in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.1.
(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of

this solution, add water to make 10 mL, then mix with 0.3 mL of a solution of sodium nitrite (1 in 100), and allow to stand for 1 minute: the solution has no more color than the following control solution.

Control solution: Dissolve 2.0 mg of Adrenaline Bitartrate RS and 90 mg of Noradrenaline Bitartrate RS in water to make exactly 10 mL. Measure exactly 1 mL of this solution, add 1.0 mL of diluted acetic acid (100) (1 in 2) and water to make 10 mL, and proceed in the same manner.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Noradrenaline, previously dried, dissolve in 50 mL of acetic acid for nonaqueous titration by warming, if necessary, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.92 mg of $C_8H_{11}NO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Noradrenaline Injection

Noradrenaline Hydrochloride Injection
Norepinephrine Hydrochloride Injection
Norepinephrine Injection

ノルアドレナリン注射液

Noradrenaline Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of *dl*-noradrenaline ($C_8H_{11}NO_3$: 169.18).

Method of preparation Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

Description Norepinephrine Injection is a clear, colorless liquid.

It gradually becomes a pale red color by light and by air.

pH: 2.3 – 5.0

Identification Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline according to the labeled amount, to each of two test tubes A and B, and add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and 10 mL of phosphate buffer solution, pH 6.5, to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

Purity **(1)** Arterenone—Measure a volume of Noradrenaline Injection, equivalent to 10 mg of Noradrenaline according to the labeled amount, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance is not more than 0.10.

(2) Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline according to the labeled amount, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

Bacterial endotoxins ⟨*4.01*⟩ Less than 300 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Noradrenaline Injection, equivalent to about 5 mg of *dl*-noradrenaline ($C_8H_{11}NO_3$), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Piper 5 mL each of the sample solution and the standard solution, add 0.2 mL each of starch TS, then add iodine TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS dropwise until a red-purple color develops, then add water to make exactly 50 mL. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 515 nm within 5 minutes as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of *dl*-noradrenaline ($C_8H_{11}NO_3$)
$= M_S \times A_T/A_S \times 0.502$

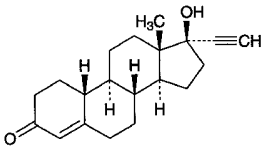
M_S: Amount (mg) of Noradrenaline Bitartrate RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Norethisterone

ノルエチステロン



$C_{20}H_{26}O_2$: 298.42
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one
[*68-22-4*]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{20}H_{26}O_2$.

Description Norethisterone occurs as a white to pale yellowish white crystalline powder. It has no odor.

It is sparingly soluble in ethanol (95), in acetone, and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

It is affected by light.

Identification **(1)** To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.

(2) To 25 mg of Norethisterone add 3.5 mL of a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate trihydrate in 25 mL of methanol. Heat under a reflux condenser on a water bath for 5 hours, cool, and add 15 mL of water. Collect the precipitate formed, wash with 1 to 2 mL of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 5 hours: the crystals melt ⟨*2.60*⟩ between 112°C and 118°C.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −32 – −37° (after drying, 0.25 g, acetone, 25 mL, 100 mm).

Melting point ⟨*2.60*⟩ 203 – 209°C.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

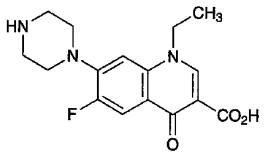
Assay Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.84 mg of $C_{20}H_{26}O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Norfloxacin

ノルフロキサシン



$C_{16}H_{18}FN_3O_3$: 319.33
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid
[*70458-96-7*]

Norfloxacin, when dried, contains not less than 99.0% of $C_{16}H_{18}FN_3O_3$.

Description Norfloxacin occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.

It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.

It is hygroscopic.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.44*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Sulfate ⟨*1.14*⟩—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, 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 0.50 mL of 0.005 mol/L sulfuric acid VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add diluted hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromophenol blue TS and water to make 50 mL (not more than 0.024%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Norfloxacin 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 <*1.11*>—Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (1:1) 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 <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 – 7 μm in particle diameter). Develop with a mixture of methanol, chloroform, toluene, diethylamine and water (20:20:10:7:4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

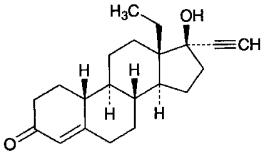
Assay Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.93 mg of $C_{16}H_{18}FN_3O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Norgestrel

ノルゲストレル



$C_{21}H_{28}O_2$: 312.45
13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one
[*6533-00-2*]

Norgestrel, when dried, contains not less than 98.0% of $C_{21}H_{28}O_2$.

Description Norgestrel occurs as white crystals or crystalline powder.

It is soluble in tetrahydrofuran and in chloroform, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

(2) Determine the infrared absorption spectrum of Norgestrel, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 206 – 212°C.

Purity **(1)** Heavy metals <*1.07*>—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution 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 30 mg of Norgestrel in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and ethyl acetate (2:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.25 mg of $C_{21}H_{28}O_2$

Containers and storage Containers—Well-closed containers.

Norgestrel and Ethinylestradiol Tablets

ノルゲストレル·エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of norgestrel ($C_{21}H_{28}O_2$: 312.45) and ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of preparation Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

Identification **(1)** Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel according to the labeled amount, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

(2) Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

(3) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel RS and 1 mg of Ethinylestradiol RS, respectively, in 10 mL of chloroform, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of *p*-toluenesulfonate in ethanol (95) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365nm): two spots from the sample solution show the similar color tone and *R*f value to each spot from the standard solutions (1) and (2).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel RS and of Ethinylestradiol RS, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

Amount (mg) of norgestrel ($C_{21}H_{28}O_2$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/100$

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/100$

M_{Sa}: Amount (mg) of Norgestrel RS
M_{Sb}: Amount (mg) of Ethinylestradiol RS

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Norgestrel and Ethinylestradiol Tablets is not less than 70%.

Start the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets, withdraw not less than 50 mL of the medium at the specified minute 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, pipet exactly V mL of the subsequent filtrate, equivalent to about 17 μg of norgestrel ($C_{21}H_{28}O_2$) and about 1.7 μg of ethinylestradiol ($C_{20}H_{24}O_2$), transfer into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent in a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel RS and about 2.5 mg of Ethinylestradiol RS, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Tb}, of norgestrel and ethinylestradiol from the sample solution, and the peak areas, A_{Sa} and A_{Sb}, of norgestrel and ethinylestradiol from the standard solution.

Dissolution rate (%) with respect to the labeled amount of norgestrel ($C_{21}H_{28}O_2$)
$= M_{Sa} \times A_{Ta}/A_{Sa} \times 1/V \times 1/C_a \times 54$

Dissolution rate (%) with respect to the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$)
$= M_{Sb} \times A_{Tb}/A_{Sb} \times 1/V \times 1/C_b \times 54$

M_{Sa}: Amount (mg) of Norgestrel RS

M_{Sb}: Amount (mg) of Ethinylestradiol RS
C_a: Labeled amount (mg) of norgestrel ($C_{21}H_{28}O_2$) in 1 tablet
C_b: Labeled amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel ($C_{21}H_{28}O_2$), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel RS and about 5 mg of Ethinylestradiol RS, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 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 <*2.01*> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

$$\text{Amount (mg) of norgestrel } (C_{21}H_{28}O_2) = M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/50$$

$$\text{Amount (mg) of ethinylestradiol } (C_{20}H_{24}O_2) = M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/50$$

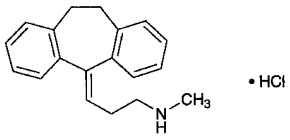
M_{Sa}: Amount (mg) of Norgestrel RS
M_{Sb}: Amount (mg) of Ethinylestradiol RS

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).
Operating conditions—
Detector: Norgestrel—An ultraviolet absorption photometer (wavelength: 241 nm).
Ethinylestradiol—A fluorophotometer (excitation wavelength: 281 nm, fluorescence wavelength: 305 nm).
Column: A stainless steel column 4.6 mm in inside 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 acetonitrile and water (11:9).
Flow rate: Adjust the flow rate so that the retention time of norgestrel 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, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is not less than 8.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

Containers and storage Containers—Tight containers.

Nortriptyline Hydrochloride

ノルトリプチリン塩酸塩



$C_{19}H_{21}N.HCl$: 299.84
3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methylpropylamine monohydrochloride
[*894-71-3*]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5% of $C_{19}H_{21}N.HCl$.

Description Nortriptyline Hydrochloride occurs as a white to yellowish white, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

The pH of a solution of Nortriptyline Hydrochloride (1 in 100) is about 5.5.

Melting point: 215 – 220°C.

Identification **(1)** To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.

(2) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 40): a red color gradually develops.

(3) Determine the absorption spectrum of a solution of Nortriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nortriptyline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Nortriptyline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Nortriptyline 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 <*1.11*>—Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform 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 <*2.03*>. Spot 4 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> 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
= 29.98 mg of $C_{19}H_{21}N.HCl$

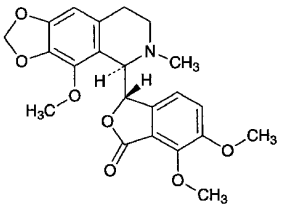
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine

Narcotine

ノスカピン



$C_{22}H_{23}NO_7$: 413.42
(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one
[*128-62-1*]

Noscapine, when dried, contains not less than 98.5% of $C_{22}H_{23}NO_7$.

Description Noscapine occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is very soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Noscapine in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noscapine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +42 – +48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting point <*2.60*> 174 – 177°C.

Purity (1) Chloride <*1.03*>—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 mL of 0.01 mol/L hydrochloric acid add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Noscapine 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) Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 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 Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (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
= 41.34 mg of $C_{22}H_{23}NO_7$

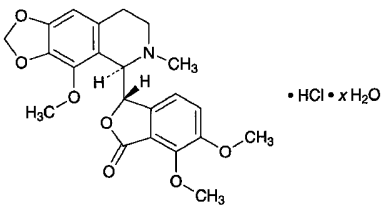
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine Hydrochloride Hydrate

Narcotine Hydrochloride

ノスカピン塩酸塩水和物



$C_{22}H_{23}NO_7.HCl.xH_2O$
(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one monohydrochloride hydrate
[*912-60-7*, anhydride]

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride $C_{22}H_{23}NO_7.HCl$: 449.88.

Description Noscapine Hydrochloride Hydrate occurs as colorless or white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in acetic acid (100), and in acetic anhydride, soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of ammonium vanadate (V) in sulfuric acid (1 in 200): an orange color is produced.

(3) Dissolve 0.02 g of Noscapine Hydrochloride Hydrate in 1 mL of water, and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

(4) Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of disodium chlomotropate dihydrate (1 in 50), and add 2 mL of sulfuric acid dropwise: a purple color is produced.

(5) Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS, and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water, and filter. Distil most of the filtrate on a water bath, add 1 mL of ethanol (99.5), and evaporate to dryness. Dry the residue at 105°C for 4 hours: the residue so obtained melts ⟨*2.60*⟩ between 174°C and 177°C.

(6) Make a solution of Noscapine Hydrochloride Hydrate (1 in 50) alkaline with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity Morphine—Dissolve 10 mg of Noscapine Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

Loss on drying ⟨*2.41*⟩ Not more than 9.0% (0.5 g, 120°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ 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
= 44.99 mg of $C_{22}H_{23}NO_7.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nystatin

ナイスタチン

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin ($C_{47}H_{75}NO_{17}$: 926.09), and one unit corresponds to 0.27 μg of nystatin ($C_{47}H_{75}NO_{17}$).

Description Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

(2) To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nystatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nystatin 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).

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Saccharomyces cerevisiae* ATCC 9763

(ii) Culture medium—Use the medium 2) Medium for test organism [12] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin RS equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

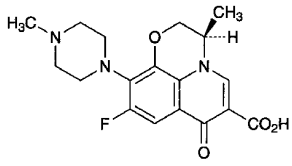
(iv) Sample solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin equivalent to about 60,000 units, dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Ofloxacin

オフロキサシン



and enantiomer

$C_{18}H_{20}FN_3O_4$: 361.37

(3*RS*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-[1,4]benzoxazine-6-carboxylic acid

[*82419-36-1*]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin ($C_{18}H_{20}FN_3O_4$).

Description Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A soluton of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 265°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ofloxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ofloxacin 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—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin obtained from the sample solution is not larger than 0.4 times the peak area of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin is not larger than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 294 nm).

Column: A stainless steel column 4.6 mm in inside 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 45°C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ofloxacin is about 20 minutes.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that from 10 μL of the standard solution.

System performance: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a

mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not less than 0.2% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate ⟨*2.50*⟩ 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
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Olive Oil

Oleum Olivae

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of *Olea europaea* Linné (*Oleaceae*).

Description Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.

It is miscible with diethyl ether, with petroleum diethyl ether and with carbon disulfide.

It is slightly soluble in ethanol (95).

The whole or a part of it congeals between 0°C and 6°C.

Congealing point of the fatty acids: 17 – 26°C

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.908 – 0.914

Acid value ⟨*1.13*⟩ Not more than 1.0.

Saponification value ⟨*1.13*⟩ 186 – 194

Unsaponifiable matters ⟨*1.13*⟩ Not more than 1.5%.

Iodine value ⟨*1.13*⟩ 79 – 88

Purity **(1)** Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5 hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in acetone to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Measure the peak heights, H_T and H_S, of methyl behenate of respective solutions: H_T is not higher than H_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Nitrogen.

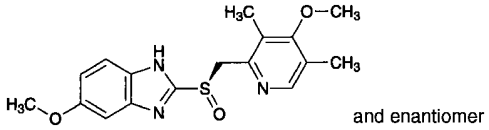
Flow rate: Adjust the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of methyl behenate obtained from 2 μL of the standard solution is 5 to 10 mm.

Containers and storage Containers—Tight containers.

Omeprazole

オメプラゾール



and enantiomer

$C_{17}H_{19}N_3O_3S$: 345.42
(*RS*)-5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl}-1*H*-benzoimidazole
[*73590-58-6*]

Omeprazole, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{19}N_3O_3S$.

Description Omeprazole occurs as a white to yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Omeprazole in *N*,*N*-dimethylformamide (1 in 25) shows no optical rotation.

It gradually turns yellowish white on exposure to light.

Melting point: about 150°C (with decomposition).

Identification **(1)** Add phosphate buffer solution, pH

7.4, to 1 mL of a solution of Omeprazole in ethanol (99.5) (1 in 1000) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Omeprazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Omeprazole in 25 mL of *N*,*N*-dimethylformamide: the solution is clear and colorless or light yellow. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 420 nm is not more than 0.3.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Omeprazole 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—Conduct the procedure soon after preparation of the sample solution. Dissolve 50 mg of Omeprazole in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each of the peak areas of the sample solution by the automatic integration method, and calculate the amounts of them by the area percentage method: each of the amount of the peaks other than omeprazole is not more than 0.1%, and the total amount of the peaks other than omeprazole is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL. If necessary, adjust the pH to 7.6 with diluted phosphoric acid (1 in 100). Add 11 volumes of acetonitrile to 29 volumes of this solution.

Flow rate: Adjust the flow rate so that the retention time of omeprazole is about 8 minutes.

Time span of measurement: About 10 times as long as the retention time of omeprazole, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the sample solution, and add the mobile phase 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 solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of omeprazole obtained from 10 μL of this solution is equivalent to 15 to 25% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Omeprazole and 25 mg of 1,2-dinitrobenzene in 5 mL of sodium borate solution (19 in 5000) and 95 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above conditions, omeprazole and 1,2-dinitrobenzene 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 of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of omeprazole is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Omeprazole, previously dried, dissolve in 70 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination using the same method on a solution consisting of 70 mL of *N*,*N*-dimethylformamide and 12 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 34.54 mg of $C_{17}H_{19}N_3O_3S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, in a cold place.

Powdered Opium

Opium Pulveratum

アヘン末

Powdered Opium is a homogeneous powder of opium obtained from *Papaver somniferum* Linné (*Papaveraceae*). Starch or Lactose Hydrate may be added.

Powdered Opium contains not less than 9.5% and not more than 10.5% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Description Powdered Opium occurs as a yellow-brown to dark brown powder.

Identification **(1)** To 0.1 g of Powdered Opium add 5 mL of diluted ethanol (7 in 10), dissolve by treating with ultrasonic waves for 10 minutes, and add diluted ethanol (7 in 10) to make 10 mL. Filter this solution, and use the filtrate as the sample solution. Separately, dissolve 25 mg of Morphine Hydrochloride Hydrate, 12 mg of Codeine Phosphate Hydrate, 2 mg of Papaverine Hydrochloride, and 12 mg of Noscapine Hydrochloride Hydrate separately in 25 mL of diluted ethanol (7 in 10), and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28)

(20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: each spot from the sample solution shows the same color tone and *R*f value of each spot obtained from the standard solution (1), the standard solution (2), the standard solution (3), and the standard solution (4) (morphine, codeine, papaverine and noscapine), respectively.

(2) To 0.1 g of Powdered Opium add 5 mL of water, and shake the mixture for 5 minutes. Filter, to the filtrate add 1 mL of a solution of hydroxylammonium chloride (3 in 10) and 1 drop of iron (III) chloride TS, and shake: a red-brown color is produced. To this solution add immediately 5 mL of diethyl ether, and shake: the diethyl ether layer has no red-purple color (meconic acid).

Loss on drying ⟨*2.41*⟩ Not more than 8.0% (1 g, 105°C, 5 hours).

Assay Place about 5 g of Powdered Opium, accurately weighed, in a mortar, and triturate it with exactly 10 mL of water. Add 2 g of calcium hydroxide and exactly 40 mL of water, and stir the mixture for 20 minutes. Filter, and shake 30 mL of the filtrate with 0.1 g of magnesium sulfate heptahydrate for 1 minute. To the mixture add 0.3 g of calcium hydroxide, shake for 1 minute, and allow to stand for 1 hour. Filter, place 20 mL of the filtrate, exactly measured, in a glass-stoppered flask, and add 10 mL of diethyl ether and 0.3 g of ammonium chloride. Shake vigorously with caution. When crystals begin to separate out, shake for 30 minutes with a mechanical shaker, and set aside overnight at a temperature of 5°C to 10°C. Decant the diethyl ether layer and filter first, and then the water layer through filter paper 7 cm in diameter. Wash the adhering crystals in the flask with three 5-mL portions of water saturated with diethyl ether, and wash the crystals on the filter paper with each of these washings. Wash the top of the glass-stoppered flask and the upper part of the filter paper with final 5 mL of water saturated with diethyl ether. Transfer the crystals and the filter paper to a beaker. Dissolve the crystals remaining in the glass-stoppered flask with the aid of 15 mL of 0.05 mol/L sulfuric acid VS, accurately measured, and pour the solution into the beaker. Wash the glass-stoppered flask with four 5-mL portions of water, and add the washings to the solution in the beaker. Titrate ⟨*2.50*⟩ the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of methyl red-methylene blue TS).

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Diluted Opium Powder

アヘン散

Diluted Opium Powder contains not less than 0.90% and not more than 1.10% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Powdered Opium	100 g
Starch or a suitable diluent	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Diluted Opium Powder occurs as a light brown powder.

Identification (1) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (1) under Powdered Opium.

(2) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (2) under Powdered Opium.

Assay Place about 50 g of Diluted Opium Powder, accurately weighed, in a glass-stoppered flask, and stir with 250 mL of dilute ethanol in a water bath at 40°C for 1 hour. Filter the mixture through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, and add 50 mL of dilute ethanol. Stir the mixture in a water bath at 40°C for 10 minutes, and filter through the same glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Evaporate the combined filtrate in a mortar to dryness on a water bath. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, and, after cooling, triturate it with exactly 10 mL of water. Proceed with this solution as directed in Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Opium Tincture

アヘンチンキ

Opium Tincture contains not less than 0.93 w/v% and not more than 1.07 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Powdered Opium	100 g
35 vol% Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of 35 vol% Ethanol.

Description Opium Tincture is a dark red-brown liquid.

It is affected by light.

Identification **(1)** To 1 mL of Opium Tincure add diluted ethanol (7 in 10) to make 10 mL, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (1) under Powdered Opium.

(2) Evaporate 1 mL of Opium Tincture to dryness on a water bath, and proceed with the residue as directed in the Identification (2) under Powdered Opium.

Alcohol number ⟨*1.01*⟩ Not less than 3.5 (Method 1).

Assay Evaporate 50 mL of Opium Tincture, accurately measured, on a water bath to dryness. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, cool, and triturate with exactly 10 mL of water. Proceed with this solution as directed in the Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Opium Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.

It contains not less than 47.0% and not more than 52.0% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

Description Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

It is colored by light.

Identification **(1)** Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codein Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution is the same in color tone and *R*f value with the corresponding spot from the standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine).

(2) A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0 and 4.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance ⟨*2.24*⟩ at 420 nm is not more than 0.20.

(2) Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminopropylsilanized silica gel for pretreatment (55 – 105 μm in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol/L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

Loss on drying ⟨*2.41*⟩ Not more than 6.0% (0.5 g, 120°C, 8 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (0.5 g).

Assay Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride for assay, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of morphine, codeine, papaverine, thebaine, narceine and noscapine, A_{T1}, A_{T2}, A_{T3}, A_{T4}, A_{T5} and A_{T6}, from the sample solution, and the peak area of morphine, A_S, from the standard solution.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$= M_S \times A_{T1}/A_S \times 0.887$

Amount (mg) of other opium alkaloids
$= M_S \times \{(A_{T2} + 0.29A_{T3} + 0.20A_{T4} + 0.19A_{T5} + A_{T6})/A_S\} \times 0.887$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

The relative retention time of codine, papaverine, thebaine, narceine and noscapine with respect to morphine obtained under the following operating conditions are as follows.

Component	Relative retention time
codeine	1.1
papaverine	1.9
thebaine	2.5
narceine	2.8
noscapine	3.6

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 morphine is about 10 minutes.

System suitability—

System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Assay Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, and dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Opium Alkaloids and Atropine Injection

アヘンアルカロイド·アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate Hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.84].

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.2 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (atropine).

Extractable volume <*6.05*> It meets the requirements.

Assay (1) Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, then add water to make 50 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethylefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (determine separately the loss on drying <*2.41*> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of

the peak area of atropine to that of the internal standard.

$$\text{Amount (mg) of atropine sulfate hydrate } [(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O] = M_S \times Q_T/Q_S \times 1/50 \times 1.027$$

M_S: Amount (mg) of Atropine Sulfate RS, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

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

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Opium Alkaloids and Scopolamine Injection

アヘンアルカロイド·スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	40 g
Scopolamine Hydrobromide Hydrate	0.6 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (scopolamine).

Extractable volume <*6.05*> It meets the requirements.

Assay (1) Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrin hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside 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: 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 morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 2 mL of Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide RS (determine separately the loss on drying <2.41> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use thus obtained solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of scopolamine to that of the internal standard.

Amount (mg) of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$)
$= M_S \times Q_T/Q_S \times 1/50 \times 1.141$

M_S: Amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine 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 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Weak Opium Alkaloids and Scopolamine Injection

弱アヘンアルカロイド·スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34) and not less than 0.027 w/v% and not more than 0.033 w/v% of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Scopolamine Hydrobromide Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Weak Opium Alkaloids and Scopolamine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28)

(200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (scopolamine).

Extractable volume ⟨*6.05*⟩ It meets the requirements.

Assay (1) Morphine—Pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$= M_S \times Q_T \times Q_S \times 0.887$

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrin hydrochloride (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 285 nm).
Column: A stainless steel column 4.6 mm in inside 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: 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 morphine 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, morphine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 4 mL of Weak Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of scopolamine to that of the internal standard.

Amount (mg) of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$)
$= M_S \times Q_T/Q_S \times 1/50 \times 1.141$

M_S: Amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.
System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine 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 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Orange Oil

Oleum Aurantii

オレンジ油

Orange Oil is the essential oil obtained by expression from the peel of the edible fruit of *Citrus* species (*Rutaceae*).

Description Orange Oil is a yellow to yellow-brown liquid.

It has a characteristic, aromatic odor, and a slightly bitter taste.

It is miscible with an equal volume of ethanol (95) with turbidity.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.472 – 1.474

Optical rotation ⟨*2.49*⟩ α_D^{20}: +85 – +99° (100 mm).

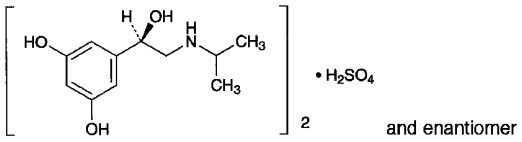
Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 0.842 – 0.848

Purity Heavy metals ⟨*1.07*⟩—Proceed with 1.0 mL of Orange Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Orciprenaline Sulfate

オルシプレナリン硫酸塩



$(C_{11}H_{17}NO_3)_2.H_2SO_4$: 520.59
5-{(1*RS*)-1-Hydroxy-2-[(1-methylethyl)amino]ethyl}benzene-1,3-diol hemisulfate
[*5874-97-5*]

Orciprenaline Sulfate contains not less than 98.5% of $(C_{11}H_{17}NO_3)_2.H_2SO_4$, calculated on the dried basis.

Description Orciprenaline Sulfate occurs as white crystals or crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.

Melting point: about 220°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Orciprenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Orciprenaline Sulfate in 10 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 Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Orciprenaline Sulfate 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) Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 328 nm is not more than 0.075.

Loss on drying ⟨*2.41*⟩ Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

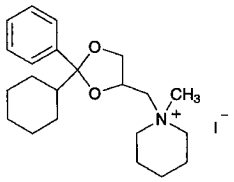
Assay Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.06 mg of $(C_{11}H_{17}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxapium Iodide

オキサピウムヨウ化物



$C_{22}H_{34}INO_2$: 471.42
1-(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-ylmethyl)-1-methylpiperidinium iodide
[*6577-41-9*]

Oxapium Iodide, when dried, contains not less than 98.5% of $C_{22}H_{34}INO_2$.

Description Oxapium Iodide occurs as a white, crystalline powder.

It is soluble in acetonitrile, in methanol and in ethanol (95), slightly soluble in water, in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Oxapium Iodide in methanol (1 in 100) does not show optical rotation.

Identification **(1)** Determine the infrared absorption spectrum of Oxapium Iodide, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver

nitrate TS: a greenish yellow precipitate is formed.

Melting point ⟨*2.60*⟩ 198 – 203°C.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Oxapium Iodide 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 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside 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 20°C to 30°C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitril, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust the flow rate so that the retention time of oxapium is about 4 minutes.

Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μL of the standard solution composes 5 to 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of oxapium beginning after the peak of iodide ion.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (9:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

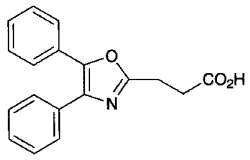
Each mL of 0.1 mol/L perchloric acid VS
= 47.14 mg of $C_{22}H_{34}INO_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Oxaprozin

オキサプロジン



$C_{18}H_{15}NO_3$: 293.32

3-(4,5-Diphenyloxazol-2-yl)propanoic acid

[*21256-18-8*]

Oxaprozin, when dried, contains not less than 98.5% of $C_{18}H_{15}NO_3$.

Description Oxaprozin occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually affected by light.

Identification Determine the infrared absorption spectrum of Oxaprozin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

Melting point ⟨*2.60*⟩ 161 – 165°C.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Oxaprozin 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 ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Oxaprozin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL and 1 mL of this solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.3% (1 g).

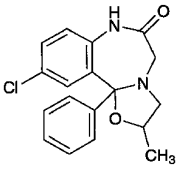
Assay Weigh accurately about 0.5 g of Oxaprozin, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.33 mg of $C_{18}H_{15}NO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxazolam

オキサゾラム



$C_{18}H_{17}ClN_2O_2$: 328.79
10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one
[*24143-17-7*]

Oxazolam, when dried, contains not less than 99.0% of $C_{18}H_{17}ClN_2O_2$.

Description Oxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Oxazolam in 10 mL of ethanol (95) by heating, and add 1 drop of hydrochloric acid: a light yellow color develops, and the solution shows 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 0.01 g of Oxazolam 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 <1.09> for primary aromatic amines.

(3) Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS, and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), 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 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt <2.60> between 96°C and 100°C.

(4) Determine the absorption spectrum of a solution of Oxazolam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Oxazolam as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color appears.

Absorbance <2.24> $E^{1\%}_{1\,cm}$ (246 nm): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

Purity **(1)** Chloride <1.03>—To 1.0 g of Oxazolam 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 <1.07>—Proceed with 1.0 g of Oxazolam 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 <1.11>—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane 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 <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1:1). Titrate <2.50> 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

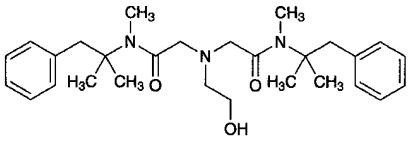
Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $C_{18}H_{17}ClN_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxethazaine

Oxetacaine

オキセサゼイン



$C_{28}H_{41}N_3O_3$: 467.64
2,2′-(2-Hydroxyethylimino)bis[*N*-(1,1-dimethyl-2-phenylethyl)-*N*-methylacetamide]
[*126-27-2*]

Oxethazaine, when dried, contains not less than 98.5% of $C_{28}H_{41}N_3O_3$.

Description Oxethazaine occurs as a white to pale yellowish white, crystalline powder.

Identification **(1)** Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxethazaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibits similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 101 – 104°C.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), 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, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Oxethazaine 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 0.40 g of Oxethazaine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, tetrahydrofuran, methanol and ammonia solution (28) (24:10:5:1) 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 sample solution are not more intense than the spot from the standard solution.

(4) 2-Aminoethanol—To 1.0 g of Oxethazaine add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

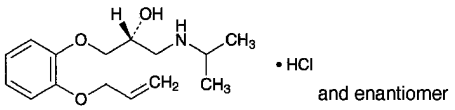
Assay Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.76 mg of $C_{28}H_{41}N_3O_3$

Containers and storage Containers—Tight containers.

Oxprenolol Hydrochloride

オクスプレノロール塩酸塩



$C_{15}H_{23}NO_3.HCl$: 301.81
(2*RS*)-1-[2-(Allyloxy)phenoxy]-3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[*6452-73-9*]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{23}NO_3.HCl$.

Description Oxprenolol Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

(2) To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Oxprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Melting point ⟨*2.60*⟩ 107 – 110°C.

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Oxprenolol 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add 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. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

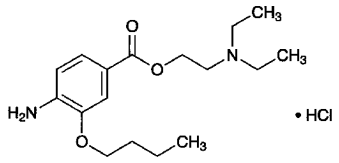
Each mL of 0.1 mol/L perchloric acid VS
= 30.18 mg of $C_{15}H_{23}NO_3.HCl$

Containers and storage Containers—Tight containers.

Oxybuprocaine Hydrochloride

Benoxinate Hydrochloride

オキシブプロカイン塩酸塩



$C_{17}H_{28}N_2O_3.HCl$: 344.88
2-(Diethylamino)ethyl 4-amino-3-butyloxybenzoate monohydrochloride
[*5987-82-6*]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0% of $C_{17}H_{28}N_2O_3.HCl$.

Description Oxybuprocaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

The pH of a solution of Oxybuprocaine Hydrochloride (1 in 10) is between 5.0 and 6.0.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Oxybuprocaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests ⟨*1.09*⟩ for primary aromatic amines.

(2) Dissolve 0.1 g of Oxybuprocaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt ⟨*2.60*⟩ between 103°C and 106°C.

(3) Determine the absorption spectrum of a solution of Oxybuprocaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Melting point ⟨*2.60*⟩ 158 – 162°C.

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Oxybuprocaine 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 0.25 g of Oxybuprocaine Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 34.49 mg of $C_{17}H_{28}N_2O_3.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Oxycodone Hydrochloride Hydrate

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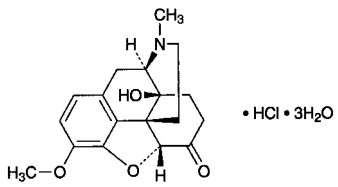


$C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87
(5*R*)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one monohydrochloride trihydrate
[*124-90-3,* anhydride]

Oxycodone Hydrochloride Hydrate contains not less than 98.0% of $C_{18}H_{21}NO_4.HCl$ (mol. wt.: 351.83), calculated on the anhydrous basis.

Description Oxycodone Hydrochloride Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.

It is affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Oxycodone Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxycodone Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −140 – −149° (0.5 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthole TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. To this solution add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge, and separate the water layer: the color of the solution is not more intense than a pale red.

(3) Codeine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and warm: no blue color is produced. Add 1 drop of nitric acid: no red color develops.

(4) Thebaine—Dissolve 0.10 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10), and heat the solution in a water bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100), and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color develops in the chloroform layer.

Water <*2.48*> 12 – 15% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 35.18 mg of $C_{18}H_{21}NO_4.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Compound Oxycodone Injection

Compound Hycodenone Injection

複方オキシコドン注射液

Compound Oxycodone Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$: 275.73).

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Compound Oxycodone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification **(1)** To 1 mL of Compound Oxycodone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

Extractable volume <*6.05*> It meets the requirement.

Assay Pipet 2 mL of Compound Oxycodone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25 \times 1.154$

Amount (mg) of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25 \times 1.070$

M_{Sa}: Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of hydrocotarnine hydrochloride for assay

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 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 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogen phosphate TS add 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order, with complete separation of these peaks.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Compound Oxycodone and Atropine Injection

Hycoato Injection

複方オキシコドン·アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$: 275.73), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification **(1)** To 1 mL of Compound Oxycodone and Atropin Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath, and dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

(4) To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the liquid is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Assay **(1)** Oxycodone hydrochloride hydrate and hydrocotarnine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Sb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25 \times 1.154$

Amount (mg) of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25 \times 1.070$

M_{Sa}: Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of hydrocotarnine hydrochloride for assay

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 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 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone hydrochloride is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarine in this order with complete separation of these peaks.

(2) Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atropine to that of the internal standards.

Amount (mg) of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$
$= M_S \times Q_T/Q_S \times 1/50 \times 1.027$

M_S: Amount (mg) of Atropine Sulfate RS, calculated on

the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 1.5 m in length, packed with 180- to 250-μm siliceous earth for gas chromatography coated with 1 to 3% of 50% phenyl-methylsilicone polymer.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

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

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide (H_2O_2: 34.01). It contains suitable stabilizers.

Description Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone.

It gradually decomposes upon standing or upon vigorous agitation.

It rapidly decomposes when in contact with oxidizing substances as well as reducing substances.

It, when alkalized, decomposes with effervescence.

It is affected by light.

pH: 3.0 – 5.0

Specific gravity d_{20}^{20}: about 1.01

Identification 1 mL of Oxydol responds to the Qualitative Tests <*1.09*> for peroxide.

Purity **(1)** Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <*1.07*>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2 mL of dilute acetic acid, 2.5 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(3) Arsenic <*1.11*>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).

(4) Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.

(5) Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

Assay Pipet 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
= 1.701 mg of H_2O_2

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

Oxygen

酸素

O_2: 32.00

Oxygen is oxygen produced by the air liquification separation method.

It contains not less than 99.5 v/v% of O_2.

Description Oxygen is a colorless gas under atmospheric pressure, and is odorless.

1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs 1.429 g.

Identification Transfer 1 mL each of Oxygen and oxygen directly from cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <*2.02*> according to the following conditions: the retention time of principal peak obtained from Oxygen is the same as that of the peak obtained from oxygen.

Operating conditions—

Proceed as directed in the operating conditions in the Purity.

Purity Nitrogen—Transfer 1.0 mL of Oxygen directly from cylinder with a pressure-reducing valve to gas-measuring tube or syringe for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with this gas as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area A_T of nitrogen. 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 gas as the standard mixed gas. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area A_S of nitrogen: A_T is not larger than A_S.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography 250- to

355-μm in particle diameter (a porosity of 0.5 nm).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

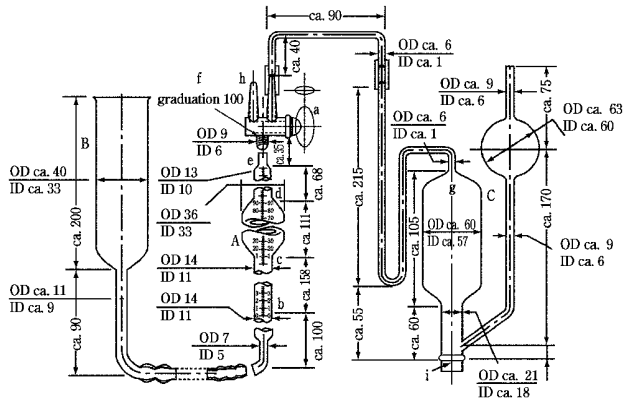
Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

System suitability—

System performance: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix thoroughly. When the test is run with 1.0 mL of the mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard mixed gas under the above operating conditions, the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Assay (i) Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b – c, d – e and e – f are graduated in 0.1 mL, and c – d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.



b–c: calibrated in 0.1 mL
c–d: calibrated in 2 mL
d–e: calibrated in 0.1 mL
e–f: calibrated in 0.1 mL
The graduations are marked with red line.
b–f: =100 mL

(ii) Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as V (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate the volume of Oxygen and V in the following formula on the basis of the gas volume at 20°C and at 101.3 kPa.

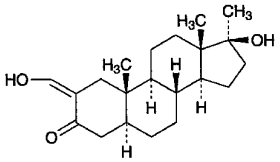
$$\text{Volume (mL) of oxygen } (O_2) = \text{volume of Oxygen (mL)} - V\text{ (mL)}$$

Containers and storage Containers—Cylinders.

Storage—Not exceeding 40°C.

Oxymetholone

オキシメトロン



$C_{21}H_{32}O_3$: 332.48

17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one

[*434-07-1*]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{32}O_3$.

Description Oxymetholone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored and decomposed by light.

Identification **(1)** Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Oxymetholone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +34 – +38° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <*2.60*> 175 – 182°C.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.

(2) Related subslances—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

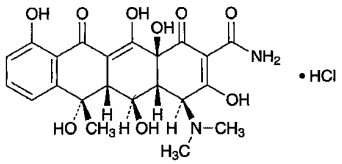
Assay Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 315 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

$$\text{Amount (mg) of } C_{21}H_{32}O_3 = A/541 \times 50{,}000$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxytetracycline Hydrochloride

オキシテトラサイクリン塩酸塩



$C_{22}H_{24}N_2O_9$.HCl: 496.89
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-Dimethylamino-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride
[*2058-46-0*]

Oxytetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 μg (potency) and not more than 945 μg (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.43).

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Oxytetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −188 – −200° (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 0.5 g of Oxytetracycline Hydrochloride 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).

(2) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of β-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as β-apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of β-apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline obtained from the sample solution are not larger than each of the peak area from the standard solution, and the total area of the peaks, α-apooxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, β-apooxytetracycline and the peaks, which appear between α-apooxytetracycline and β-apooxytetracycline, is not larger than the peak area of β-apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak, obtained from the sample solution is not larger than 4 times the peak area of 4-epioxytetracycline from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene

copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of *t*-butanol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of *t*-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	70 → 10	30 → 90
20 – 35	10 → 20	90 → 80

Flow rate: 1.0 mL/min.

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α-apooxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epioxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β-apooxytetracycline stock solution and 6 mL of α-apooxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-epioxytetracycline, oxytetracycline, tetracycline, α-apooxytetracycline and β-apooxytetracycline are eluted in this order with the resolutions between the peaks, 4-epioxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and α-apooxytetracycline and β-apooxytetracycline being not less than 4, not less than 5 and not less than 4, respectively, and the symmetry factor of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epioxytetracycline is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of oxytetracycline.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Oxytetracycline Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline 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 theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

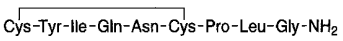
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Oxytocin

オキシトシン



$C_{43}H_{66}N_{12}O_{12}S_2$: 1007.19
[*50-56-6*]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the dehydrated and de-acetic acid basis.

Description Oxytocin occurs as a white powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It dissolves in hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.

It is hygroscopic.

Identification Determine the absorption spectrum of a solution of Oxytocin (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent amino acids Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, seal the tube under reduced pressure, and heat at 110 to 115°C for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the respective molar ratios with respect to leucine: 0.95 – 1.05 for aspartic acid, 0.95 – 1.05 for glutamic acid, 0.95 – 1.05 for proline, 0.95 – 1.05 for glycine, 0.80 – 1.10 for isoleucine, 0.80 – 1.05 for tyrosine and 0.80 – 1.05 for cystine, and not more than 0.01 each for others.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B and C according to the following table.

Mobile phase	A	B	C
Citric acid monohydrate	19.80 g	22.00 g	6.10 g
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g
Sodium chloride	5.66 g	7.07 g	54.35 g
Ethanol (99.5)	260.0 mL	20.0 mL	—
Benzyl alcohol	—	—	5.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL
Capryric acid	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	2000 mL	1000 mL	1000 mL
pH	3.3	3.2	4.9

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 9	100	0	0
9 – 25	0	100	0
25 – 61	0	100 → 0	0 → 100
61 – 80	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 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 more than 30 minutes while passing Nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the

peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

Purity **(1)** Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 less than 6.0% and not more than 10.0%.

$$\text{Amount (\%) of acetic acid } (C_2H_4O_2) = M_S/M_T \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of acetic acid (100)
M_T: Amount (mg) of the sample

Internal standard solution—A solution of propionic acid in the mobile phase (1 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside 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: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, 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 acetic acid 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, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetic acid to that of the internal standard is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than Oxytocin is not more than 1.5%, and the total of them is not more than 5.0%.

Operating conditions—
Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 2.5 times as long as the retention time of oxytocin.
System suitability—
Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 μL of this solution is equivalent to 5 to 15% of that from 50 μL of the solution for system suitability test.
System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.
System repeatability: When the test is repeated 6 times with 50 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

Water <*2.48*> Not more than 5.0% (50 mg, coulometric titration).

Assay Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin RS in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of oxytocin.

$$\text{Units per mg of Oxytocin, calculated on the dehydrated and de-acetic acid basis} = M_S/M_T \times A_T/A_S \times 100$$

M_S: Units per mL of the standard solution
M_T: Amount (mg) of sample, calculated on the dehydrated and de-acetic acid basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside 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: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.
Mobile phase B: A mixture of water and acetonitrile (1:1).
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	70 → 40	30 → 60
30 – 30.1	40 → 70	60 → 30
30.1 – 45	70	30

Flow rate: About 1.0 mL per minute.

System suitability—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 20 mL of the mobile phase A. When the procedure is run with 25 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At 2 to 8°C.

Oxytocin Injection

オキシトシン注射液

Oxytocin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

Method of preparation Prepare as directed under Injections, with Oxytocin.

Description Oxytocin Injection is a colorless, clear liquid.

pH ⟨*2.54*⟩ 2.5 – 4.5

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/oxytocin Unit.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to the Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin RS in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each mL contains about 1 Unit, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of oxytocin.

$$\text{Units per mL of Oxytocin Injection} = M_S \times A_T/A_S \times b/a$$

M_S: Units per mL of the standard solution

a: Volume (mL) of sample

b: Total volume of the sample solution prepared by diluting with the diluent

Diluent: Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	70 → 40	30 → 60
30 – 30.1	40 → 70	60 → 30
30.1 – 45	70	30

Flow rate: About 1.0 mL per minute.

System suitability—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 100 mL of the mobile phase A. When the procedure is run with 100 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

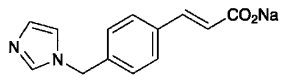
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Ozagrel Sodium

オザグレルナトリウム



$C_{13}H_{11}N_2NaO_2$: 250.23
Monosodium (2*E*)-3-[4-(1*H*-imidazol-1-ylmethyl)phenyl]prop-2-enoate
[*189224-26-8*]

Ozagrel Sodium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{13}H_{11}N_2NaO_2$.

Description Ozagrel Sodium occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Ozagrel Sodium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ozagrel Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ozagrel Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ozagrel Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ozagrel Sodium (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt.

pH <*2.54*> The pH of a solution prepared by dissolving 0.5 g of Ozagrel Sodium in 10 mL of water is between 9.5 and 10.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Ozagrel Sodium in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 2.0 g of Ozagrel Sodium in 30 mL of water, add 1 mL of acetic acid (100) and water to make 50 mL, shake, and allow to stand for 30 minutes. Filter the solution, discard the first 5 mL of the filtrate, and to 25 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with 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 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Ozagrel 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).

(4) Related substances—Dissolve 50 mg of Ozagrel Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: each of the amount other than ozagrel is not more than 0.2%, and the total amount other than ozagrel is not more than 0.5%.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 2 times as long as the retention time of ozagrel, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ozagrel obtained from 5 μL of this solution is equivalent to 15 to 25% of that from 5 μL of the solution for system suitability test.

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 and the symmetry factor of the peak of ozagrel are not less than 6000 and not more than 2.0, respectively.

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

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 25 mg each of Ozagrel Sodium and Ozagrel Sodium RS, both previously dried, and dissolve each in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ozagrel to that of the internal standard.

$$\text{Amount (mg) of } C_{13}H_{11}N_2NaO_2 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Ozagrel Sodium RS

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside 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 acetate (3 in 1000) and methanol (4:1).

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

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating condi-

tions, the internal standard and ozagrel are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of ozagrel is not more than 2.0.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ozagrel to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ozagrel Sodium for Injection

注射用オザグレルナトリウム

Ozagrel Sodium for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$: 250.23).

Method of preparation Prepare as directed under Injections, with Ozagrel Sodium.

Description Ozagrel Sodium for Injection occurs as white masses or powder.

Identification Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 40 mg of Ozagrel Sodium according to the labeled amount, in water to make 40 mL. To 1 mL of this solution add water to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 269 nm and 273 nm.

pH Being specified separately.

Purity Related substances—Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 0.20 g of Ozagrel Sodium according to the labeled amount, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

Bacterial endotoxins <*4.01*> Less than 3.7 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve an amount of Ozagrel Sodium for Injection, equivalent to about 0.4 g of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$), in water to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and 5 mL of water, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium RS, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ozagrel Sodium.

$$\text{Amount (mg) of ozagrel sodium } (C_{13}H_{11}N_2NaO_2) = M_S \times Q_T/Q_S \times 16$$

M_S: Amount (mg) of Ozagrel Sodium RS

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Containers and storage Containers—Hermetic containers.

Pancreatin

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

Description Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

Purity **(1)** Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

(2) Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue does not exceed 20 mg.

Loss on drying <*2.41*> Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <*2.44*> Not more than 5% (1 g).

Assay **(1)** Starch digestive activity <*4.03*>

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in 1.1. Measurement of starch saccharifying activity of 1. Assay for starch digestive activity under Digestion Test.

(2) Protein digestive activity <*4.03*>

(i) Substrate solution—Use the substrate solution 2 described in (2) Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir,

and add ice-cold water to make exactly 200 mL.

(iii) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test, using trichloroacetic acid TS B as the precipitation reagent.

(3) Fat digestive activity <*4.03*>

(i) Emulsifier—Prepare with 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II as directed in (3) Assay for fat digestive activity under Digestion Test.

(ii) Substrate solution—Use the substrate solution described in (3) Assay for fat digestive activity under the Digestion Test.

(iii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL.

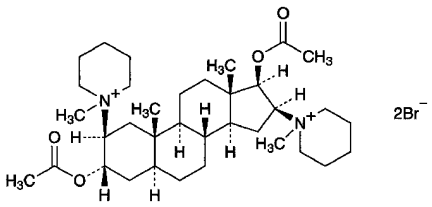
(iv) Procedure—Proceed as directed in (3) Assay for fat digestive activity under Digestion Test, using phosphate buffer solution, pH 8.0, as the buffer solution.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Pancuronium Bromide

パンクロニウム臭化物



$C_{35}H_{60}Br_2N_2O_4$: 732.67

1,1′-(3α,17β-Diacetoxy-5α-androstan-2β,16β-diyl)bis(1-methylpiperidinium) dibromide

[*15500-66-0*]

Pancuronium Bromide contains not less than 98.0% and not more than 102.0% of $C_{35}H_{60}Br_2N_2O_4$, calculated on the dehydrated basis.

Description Pancuronium Bromide occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride.

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Pancuronium Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for bromide.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +38 – +42° (0.75 g calculated on the dehydrated basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

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

(2) Related substances—Dissolve 50 mg of Pancuronium Bromide in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, weigh exactly 5 mg of dacuronium bromide for thin-layer chromatography, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly potassium bismuth iodide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

Water <*2.48*> Not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

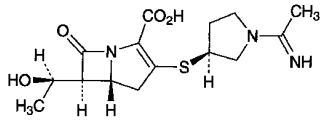
Each mL of 0.1 mol/L perchloric acid VS
= 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Panipenem

パニペネム



$C_{15}H_{21}N_3O_4S$: 339.41

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-[(3*S*)-1-(1-iminoethyl)pyrrolidin-3-ylsulfanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

[*87726-17-8*]

Panipenem contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis and corrected on the amount of the residual solvent. The potency of Panipenem is expressed as mass (potency) of panipenem ($C_{15}H_{21}N_3O_4S$).

Description Panipenem occurs as a white to light yellow, crystalline powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is hygroscopic.

It deliquesces in the presence of moisture.

Identification **(1)** Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxylammonium chloride-ethanol 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) Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 296 nm and 300 nm.

(3) Determine the infrared absorption spectrum of Panipenem as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1760 cm^{-1}, 1676 cm^{-1}, 1632 cm^{-1}, 1588 cm^{-1}, 1384 cm^{-1} and 1249 cm^{-1}.

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (298 nm): 280 – 310 (50 mg calculated on the anhydrous and desolvent basis, 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 2500 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +55 – +65° (0.1 g, calculated on the anhydrous and corrected on the amount of the residual solvent, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Being specified separately.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Panipenem 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) Residual solvents <*2.46*>—Weigh accurately about 0.2 g of Panipenem, transfer to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, add water to make exactly 200 mL. Pipet 1 mL and 2 mL of this solution, and add water to them to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use these solutions as the standard solution (1) and the standard solution (2). Shake gently the sample solution and the standard solutions (1) and (2) in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the gas in each container as directed under Gas Chromatography <*2.02*> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of ethanol and acetone to that of the internal standard from the sample solution, the ratios, Q_{Sa1} and Q_{Sb1}, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (1), and the ratios, Q_{Sa2} and Q_{Sb2}, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (2). Calculate the amount of the ethanol and acetone by the following formula: ethanol is not more than 5.0% and acetone is not more than 1.0%.

Amount (%) of ethanol in Panipenem
$= 15 \times 0.79 \times (Q_{Ta} + Q_{Sa2} - 2Q_{Sa1})/2(Q_{Sa2} - Q_{Sa1}) \times 1/1000 \times 100/M$

M: Amount (g) of Panipenem

Amount (%) of acetone in Panipenem
$= 3 \times 0.79 \times (Q_{Tb} + Q_{Sb2} - 2Q_{Sb1})/2(Q_{Sb2} - Q_{Sb1}) \times 1/1000 \times 100/M$

M: amount (g) of Panipenem
0.79: Specific gravity (d_{20}^{20}) of ethanol (99.5) and acetone

Internal standard solution—A solution of 1-propanol (1 in 400).

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A glass column 1 mm in inside diameter and 40 m in length, coated with porous polymer beads for gas chromatography.

Column temperature: A constant temperature of about 140°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 mL of the gas of the standard solution (2) under the above operating conditions, ethanol, acetone and the internal standard are eluted in this order with the resolution between ethanol and acetone being not less than 4.

System repeatability: When the test is repeated 6 times with 1 mL of the gas of the standard solution (2) 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 5.0%.

(4) Related substances—Being specified separately.

Water <*2.48*> Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 μL of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <*2.02*> according to the following condition, and calculate the ratios, Q_T, Q_{S1} and Q_{S2} of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not more than 5.0%.

Amount of water (%)
$= M_S/M_T \times (Q_T + Q_{S2} - 2Q_{S1})/2(Q_{S2} - Q_{S1}) \times 1/100 \times 100$

M_S: Amount (g) of water
M_T: Amount (g) of Panipenem

Internal standard solution—A solution of acetonitrile in methanol (1 in 100).
Operating conditions—
Detector: A thermal conductivity detector.
Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature of about 125°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.
System suitability—
System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted in this order with the resolution between water and internal standard being not less than 10.
System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of water to that of the internal standard is not more than 5.0%.

Residue on ignition Being specified separately.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.15 EU/mg (potency).

Assay Weigh accurately an amount of Panipenem and Panipenem RS, equivalent to about 0.1 g (potency), dissolve separately in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test within 30 minutes after preparation of the solutions with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of panipenem to that of the internal standard.

Amount [μg (potency)] of panipenem ($C_{15}H_{21}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 1000$

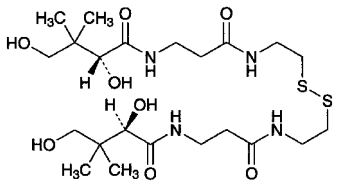
M_S: Amount [mg (potency)] of Panipenem RS

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 8.0 and acetonitrile (50:1).
Flow rate: Adjust the flow rate so that the retention time of the internal standard 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, panipenem 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.
Storage—Not exceeding −10°C.

Pantethine

パンテチン



$C_{22}H_{42}N_4O_8S_2$: 554.72
Bis(2-{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoylamino}ethyl) disulfide
[*16816-67-4*]

Pantethine is an aqueous solution containing 80% of pantethine.

Pantethine contains not less than 98.0% of pantethine ($C_{22}H_{42}N_4O_8S_2$), calculated on the anhydrous basis.

Description Pantethine is a clear, colorless to pale yellow viscous liquid.
It is miscible with water, with methanol and with ethanol (95).
It is decomposed by light.

Identification **(1)** To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.
(2) To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.
(3) To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +15.0 – +18.0° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pantethine 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample 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 ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with 2-butanone saturated with water to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Mercapto compounds—To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red color is not developed.

Water ⟨*2.48*⟩ 18 – 22% (0.2 g, volumetric titration, direct titration).

Residue on Ignition ⟨*2.44*⟩ Not more than 0.1% (2 g).

Assay Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

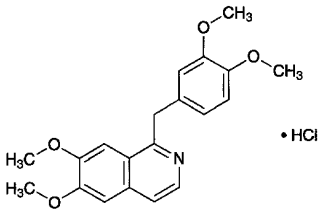
Each mL of 0.05 mol/L bromine VS
= 5.547 mg of $C_{22}H_{42}N_4O_8S_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 10°C.

Papaverine Hydrochloride

パパベリン塩酸塩



$C_{20}H_{21}NO_4.HCl$: 375.85
6,7-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline monohydrochloride
[*61-25-6*]

Papaverine Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{21}NO_4.HCl$.

Description Papaverine Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

Identification **(1)** To 1 mg of Papaverine Hydrochloride add 1 drops of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes to deep red, then to brown.

(2) Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts ⟨*2.60*⟩ between 145°C and 148°C.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

(3) Readily carbonizable substances <*1.15*>—Perform the test with 0.12 g of Papaverine Hydrochloride: the solution has no more color than Matching Fluid for Color S or P.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Papaverine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <*2.50*> 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
= 37.59 mg of $C_{20}H_{21}NO_4$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Papaverine Hydrochloride Injection

パパベリン塩酸塩注射液

Papaverine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of papaverine hydrochloride ($C_{20}H_{21}NO_4$.HCl: 375.85).

Method of preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.
pH: 3.0 – 5.0

Identification **(1)** To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine Hydrochloride according to the labeled amount, with water to 10 mL, render the solution alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 3 hours: the residue so obtained melts <*2.60*> between 145°C and 148°C.

(3) Proceed with 1 mg each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

(4) Alkalify 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off, and acidity the filtrate with dilute nitric acid: the solution responds to Qualitative Tests <*1.09*> (2) for chloride.

Bacterial endotoxins <*4.01*> Less than 6.0 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dilute an exactly measured volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride ($C_{20}H_{21}NO_4$.HCl), with water to 10 mL, render the solution alkaline with ammonia TS, and extract with 20-mL, 15-mL, 10-mL and 10-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and re-extract the washings with two 5-mL portions of chloroform. Combine all the chloroform extracts, and distil the chloroform on a water bath. Dissolve the residue in 30 mL of acetic acid (100), and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.79 mg of $C_{20}H_{21}NO_4$.HCl

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Paraffin

パラフィン

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

Description Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

It is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity d_{20}^{20}: about 0.92 (proceed as directed in 4.2. in 4. Specific gravity under Fats and Fatty Oils Test <*1.13*>).

Identification **(1)** Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Melting point <*2.60*> 50 – 75°C (Method 2).

Purity **(1)** Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is produced.

(2) Heavy metals <*1.07*>—Ignite 2.0 g of Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g

of Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(4) Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer.

(5) Readily carbonizable substances—Melt 5.0 g of Paraffin placed in a Nessler tube at a temperature near the melting point. Add 5 mL of sulfuric acid for readily carbonizable substances, and warm at 70°C for 5 minutes in a water bath. Remove the tube from the water bath, immediately shake vigorously and vertically for 3 seconds, and warm for 1 minute in a water bath at 70°C. Repeat this procedure five times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobalt (II) Chloride CS, 0.5 mL of Copper (II) Sulfate CS and 5 mL of liquid paraffin to 3.0 mL of Iron (III) Chloride CS, and shake vigorously.

Containers and storage Containers—Well-closed containers.

Liquid Paraffin

流動パラフィン

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petrolatum.

Tocopherols of a suitable form may by added at a concentration not exceeding 0.001% as a stabilizer.

Description Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

Identification (1) Heat Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.860 – 0.890

Viscosity ⟨*2.53*⟩ Not less than 37 mm^2/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals ⟨*1.07*⟩—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS

with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage Containers—Tight containers.

Light Liquid Paraffin

軽質流動パラフィン

Light Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

Identification **(1)** Heat Light Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.830 – 0.870

Viscosity ⟨*2.53*⟩ Less than 37 mm²/s (Method 1, 37.8°C).

Purity **(1)** Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals ⟨*1.07*⟩—Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Light Liquid Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage Containers—Tight containers.

Paraformaldehyde

パラホルムアルデヒド

$(CH_2O)_n$
Poly(oxymethylene)
[*30525-89-4*]

Paraformaldehyde contains not less than 95.0% of CH_2O: 30.03.

Description Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in hot water, in hot dilute hydrochloric acid, in

sodium hydroxide TS and in ammonia TS.

It sublimes at about 100°C.

Identification **(1)** Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

(2) Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

(2) Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

(3) Chloride <*1.03*>—Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize with diluted nitric acid (3 in 10), 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 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

(4) Sulfate <*1.14*>—Dissolve 1.5 g of Paraformaldehyde in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at abut 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, 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 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.011%).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, and titrate <*2.50*> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH_2O

Containers and storage Containers—Tight containers.

Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

Method of preparation

Paraformaldehyde, finely powdered	35 g
Procaine Hydrochloride, finely powdered	35 g
Hydrous Lanolin	a sufficient quantity
	To make 100 g

Prepare as directed under Ointments, with the above ingredients.

Description Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

Identification **(1)** To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

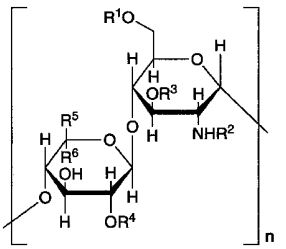
(2) To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests <*1.09*> for primary aromatic amines (procaine hydrochloride).

(3) To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Seperately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same *R*f value.

Containers and storage Containers—Tight containers.

Parnaparin Sodium

パルナパリンナトリウム



R^1, R^3, R^4 = SO_3Na or H

R^2 = SO_3Na or $-NH-C(=O)-CH_3$

R^5 = CO_2Na, R^6 = H

or

R^5 = H, R^6 = CO_2Na

n = 4–21

Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and with copper (II) acetate, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

Description Parnaparin Sodium occurs as a white or light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

(2) A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests <*1.09*> for sodium salt.

pH <*2.54*> Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Parnaparin 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).

Loss on drying <*2.41*> Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Molecular mass Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6400.

(i) Creation of calibration curve—Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak height, H_{UV}, in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, H_{RI}, in chromatogram obtained by the differential refractometer. Calculate the ratio of H_{UV} to H_{RI}, H_{RI}/H_{UV}, at each peak. Assume the molecular mass in the 4th peak from the low molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the H_{RI}/H_{UV} at the corresponding peak. Make the calculation to multiply the H_{RI}/H_{UV} at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect two stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

Column temperature; A constant temperature of about 40°C.

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than ten peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram (H_{UV} and H_{RI}) is not more than 3.0%.

(ii) Determination of molecular mass—Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Divide the main peak observed between 30 min and 45 min to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

$$\text{Mean molecular mass of parnaparin sodium} = \Sigma(n_i \cdot M_i)/\Sigma n_i$$

n_i: The differential refractometer strength of fraction i in the main peak of chromatogram
M_i: Molecular mass of fraction i in main peak

Operating conditions—
Detector: A differential refractometer.
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in (i) Creation of calibration curve.
System suitability—
Proceed as directed in (i) Creation of calibration curve.

Distribution of molecular mass The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is calculated by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

$$\text{Distribution of molecular mass (\%)} = (\Sigma n_j / \Sigma n_i) \times 100$$

n_i: The differential refractometer strength of fraction i in the main peak of chromatogram
Σn_j: Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

The degree of sulfate ester Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate <*2.50*> with 0.1 mol/L Sodium hydroxide VS (potentiometric titration). Calculate the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester
= the first equivalence point (mL)/[the second equivalence point (mL) – first equivalence point (mL)]

Total nitrogen Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination <*1.08*>: it contains not less than 1.9% and not more than 2.3% of nitrogen (N:14.01).

Anti-factor IIa activity Determine the potency of anti-factor IIa activity of Parnaparin Sodium according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per milligram calculated with reference to the dried substance.

(i) Standard solution Dissolve Low-molecular Mass Heparin RS with isotonic sodium chloride solution to make solutions which contain 0.1, 0.2 and 0.3 low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To each tube add 0.10 mL of human normal plasma and mix, and incubate at 37 ± 1°C accurately for 1 minute. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at 37 ± 1°C, and after the mixing incubate accurately for 5 minutes at 37 ± 1°C. Then, to each tube add 0.10 mL of sodium calcium solution (277 in 100,000) which is pre-warmed at 37 ± 1°C, mix, start a stop watch simultaneously, and permit to stand at the same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium
= the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution × b/a

a: Amount (mg) of Parnaparin Sodium
b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

The ratio of anti-factor Xa activity to anti-factor IIa activity Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

Assay

(i) Standard solution Dissolve Low-molecular Mass-Heparin RS in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of either the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at 37 ± 1°C. Next, to each tube add 0.10 mL of facter Xa TS and mix it, permit to stand 37 ± 1°C accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 min at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.2 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution obtained from this solution as the blank.

(iv) Calculation method Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution

using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium
= the low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mL of the sample solution × b/a

a: Amount (mg) of Parnaparin Sodium
b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

Container and Storage
Container—Well-closed containers.

Peanut Oil

Oleum Arachidis

ラッカセイ油

Peanut Oil is the fixed oil obtained from the seeds of *Arachis hypogaea* Linné (*Leguminosae*).

Description Peanut Oil is a pale yellow, clear oil. It is odorless or has a slight odor. It has a mild taste.
It is miscible with diethyl ether and with petroleum ether.
It is slightly soluble in ethanol (95).
Specific gravity d_{25}^{25}: 0.909 – 0.916
Congealing point of the fatty acids: 22 – 33°C

Identification Saponify 5 g of Peanut Oil by boiling with 2.5 mL of sodium hydroxide solution (3 in 10) and 12.5 mL of ethanol (95). Evaporate the ethanol, dissolve the residue in 50 mL of hot water, and add dilute hydrochloric acid in excess until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids, and dissolve them in 75 mL of diethyl ether. To the diethyl ether solution add a solution of 4 g of lead (II) acetate trihydrate in 40 mL of ethanol (95), and allow the mixture to stand for 18 hours. Filter the supernatant liquid, transfer the precipitate to the filter with the aid of diethyl ether, and filter by suction. Place the precipitate in a beaker, heat it with 40 mL of dilute hydrochloric acid and 20 mL of water until the oily layer is entirely clear, cool, and decant the water layer. Boil the fatty acids with 50 mL of diluted hydrochloric acid (1 in 100). When the solution prepared by dissolving 0.1 g of the fatty acids in 10 mL of ethanol (95) is not darkened by the addition of 2 drops of sodium sulfide TS, allow the fatty acids to solidify, and press them between dry filter papers to exclude moisture. Dissolve the solid fatty acid in 25 mL of diluted ethanol (9 in 10) with the aid of gentle heat, and then cool to 15°C to crystallize the fatty acids. Recrystallize them from diluted ethanol (9 in 10) and dry in a desiccator (phosphorus (V) oxide, in vacuum) for 4 hours: the melting point ⟨*1.13*⟩ of the dried crystals is between 73°C and 76°C.

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 188 – 196

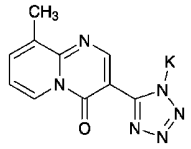
Unsaponifiable matters ⟨*1.13*⟩ Not more than 1.5%.

Iodine value ⟨*1.13*⟩ 84 – 103

Containers and storage Containers—Tight containers.

Pemirolast Potassium

ペミロラストカリウム



$C_{10}H_7KN_6O$: 266.30
Monopotassium 5-(9-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-1*H*-tetrazol-1-ide
[*100299-08-9*]

Pemirolast Potassium contains not less than 98.5% and not more than 101.0% of $C_{10}H_7KN_6O$, calculated on the anhydrous basis.

Description Pemirolast Potassium occurs as a light yellow crystalline powder.
It is freely soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).
It dissolves in potassium hydroxide TS.
Melting point: about 322°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Pemirolast Potassium in diluted potassium hydroxide TS (1 in 10,000) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pemirolast Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Pemirolast Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Pemirolast Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3) Pemirolast Potassium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for potassium salt.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.5 g of Pemirolast Potassium in 10 mL of water is clear and colorless.
(2) Heavy metals ⟨*1.07*⟩—Proceed with 0.5 g of Pemirolast Potassium 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).
(3) Related substances—Dissolve 50 mg of Pemirolast Potassium in 50 mL of a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 100 mL. To exactly 2.5 mL of this solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as

directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than pemirolast obtained from the sample solution is not larger than the peak area of pemirolast 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.

Time span of measurement: About 9 times as long as the retention time of pemirolast.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 25 mL. Confirm that the peak area of pemirolast obtained with 10 μL of this solution is equivalent to 15 to 25% 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 pemirolast are not less than 3000 and not more than 1.7, respectively.

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

(4) Residual solvent—Being specified separately.

Water <2.48> Not more than 0.5% (0.1 g, coulometric titration).

Assay Weigh accurately about 50 mg each of Pemirolast Potassium and Pemirolast Potassium RS (separately determine the water <2.48> in the same manner as Pemirolast Potassium), dissolve in a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make them exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution to each, then add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pemirolast to that of the internal standard.

$$\text{Amount (mg) of } C_{10}H_7KN_6O = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust the flow rate so that the retention time of pemirolast 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, pemirolast 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pemirolast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Pemirolast Potassium for Syrup

シロップ用ペミロラストカリウム

Pemirolast Potassium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Preparations for Syrups, with Pemirolast Potassium.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 255 nm and 259 nm and between 355 nm and 359 nm.

pH Being specified separately.

Uniformity of dosage units <6.02> Perform the test according to the following method: Pemirolast Potassium for Syrup in single-unit containers meet the requirement of the Content uniformity test.

Dissolve the total amount of the content of 1 container of Pemirolast Potassium for Syrup in water to make exactly V mL so that each mL contains about 50 μg of pemirolast potassium ($C_{10}H_7KN_6O$). Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of pemirolast potassium } (C_{10}H_7KN_6O) = M_S \times A_T/A_S \times V/400$$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Assay Powder Pemirolast Potassium for Syrup. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water <2.48> in the same manner as Pemirolast Potassium), 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 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Pemirolast Potassium Tablets

ペミロラストカリウム錠

Pemirolast Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Tablets, with Pemirolast Potassium.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pemirolast Potassium Tablets add 50 mL of water for 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), and shake to disintegrate the tablet completely. Then, add water to make exactly V mL so that each mL contains about 50 μg of pemirolast potassium ($C_{10}H_7KN_6O$), and filter. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times A_T/A_S \times V/400$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 5.0 as the dissolution medium, the dissolution rate in 45 minutes of a 5-mg tablet is not less than 75%, and that in 60 minutes of a 10-mg tablet is not less than 70%.

Start the test with 1 tablet of Pemirolast Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, and add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of pemirolast potassium ($C_{10}H_7KN_6O$) according to the labeled amount. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Pemirolast Potassium RS (separately determine the water <*2.48*> in the same manner as Pemirolast Potassium), dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the standard solution. Then, proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

C: Labeled amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Pemirolast Potassium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), add 50 mL of water, shake thoroughly for 20 minutes, then add water to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water <*2.48*> in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T sand A_S, at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

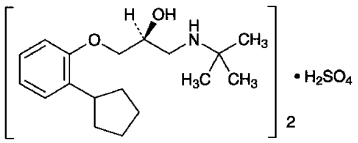
Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Penbutolol Sulfate

ペンブトロール硫酸塩



$(C_{18}H_{29}NO_2)_2.H_2SO_4$: 680.94
(2*S*)-3-(2-Cyclopentylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol hemisulfate
[*38363-32-5*]

Penbutolol Sulfate, when dried, contains not less than 98.5% of $(C_{18}H_{29}NO_2)_2.H_2SO_4$.

Description Penbutolol Sulfate occurs as a white crystalline powder.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride and in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Penbutolol Sulfate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Penbutolol Sulfate, previously dried, as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to Qualitative Tests <*1.09*> for sulfate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -23 – $-25°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

Melting point <*2.60*> 213 – 217°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Penbutolol Sulfate 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 <*1.11*>—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 10 μL each of the sample 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-propanol, ethanol (95) and ammonia solution (28) (85:12:3) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

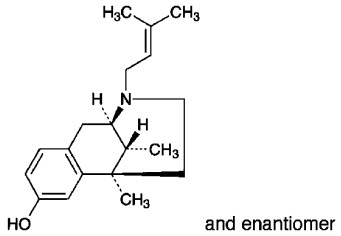
Assay Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.09 mg of $(C_{18}H_{29}NO_2)_2.H_2SO_4$

Containers and storage Containers—Well-closed containers.

Pentazocine

ペンタゾシン



and enantiomer

$C_{19}H_{27}NO$: 285.42
(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzoazocin-8-ol
[*359-83-1*]

Pentazocine, when dried, contains not less than 99.0% of $C_{19}H_{27}NO$.

Description Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in diethyl ether and practically insoluble in water.

Identification **(1)** To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

(2) Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

(3) Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).

Melting point ⟨*2.60*⟩ 150 – 158°C

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Pentazocine 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Pentazocine according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

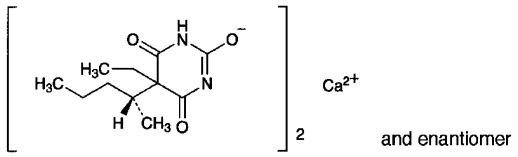
Assay Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.54 mg of $C_{19}H_{27}NO$

Containers and storage Containers—Well-closed containers.

Pentobarbital Calcium

ペントバルビタールカルシウム



$C_{22}H_{34}CaN_4O_6$: 490.61
Monocalcium bis[5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidin-2-olate]
[*76-74-4*, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of $C_{22}H_{34}CaN_4O_6$, calculated on the dried basis.

Description Pentobarbital Calcium occurs as a white powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

Identification **(1)** Determine the infrared absorption spectrum of Pentobarbital Calcium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests ⟨*1.09*⟩ (1), (2) and (3) for calcium salt.

Purity **(1)** Chloride ⟨*1.03*⟩—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to 15 mL of the subsequent 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 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Heavy metals ⟨*1.07*⟩—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 80 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to 40 mL of the subsequent filtrate add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, 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 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then 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).

(3) Related substances—Dissolve 10 mg of Pentobarbital Calcium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not larger than 3/10 times the peak area of pentobarbital from the standard solution, and the total of these peak area is not larger than the peak area of pentobarbital 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.

Time span of measurement: About 3 times as long as the

retention time of pentobarbital beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 μL of this solution is equivalent to 5 to 15% of that from 20 μL of the standard solution.

System performance: Proceed as directed in the system performance in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pentobarbital is not more than 5%.

Loss on drying ⟨*2.41*⟩ Not more than 7.0% (1 g, 105°C, 5 hours).

Assay Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pentobarbital to that of the internal standard.

$$\text{Amount (mg) of } C_{22}H_{34}CaN_4O_6 = M_S \times Q_T/Q_S \times 1.084$$

M_S: Amount (mg) of Pentobarbital RS

Internal standard solution—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitorile, and add water to make 100 mL.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitorile.

Flow rate: Adjust the flow rate so that the retention time of pentobarbital 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, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

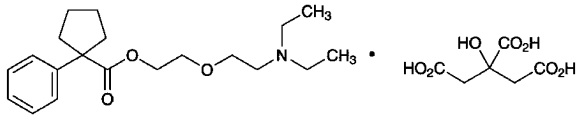
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pentoxyverine Citrate

Carbetapentane Citrate
Carbetapentene Citrate

ペントキシベリンクエン酸塩



$C_{20}H_{31}NO_3.C_6H_8O_7$: 525.59

2-[2-(Diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate monocitrate

[*23142-01-0*]

Pentoxyverine Citrate, when dried, contains not less than 98.5% of $C_{20}H_{31}NO_3.C_6H_8O_7$.

Description Pentoxyverine Citrate occurs as a white, crystalline powder.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 0.1 g of Pentoxyverine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Pentoxyverine Citrate, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pentoxyverine Citrate (1 in 10) responds to Qualitative Tests ⟨*1.09*⟩ (1) and (2) for citrate.

Melting point ⟨*2.60*⟩ 92 – 95°C

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pentoxyverine 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Pentoxyverine Citrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentoxyverine Citrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 ⟨*2.03*⟩. Spot 15 μL each of the sample solution and standard solution on a

plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28) (25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

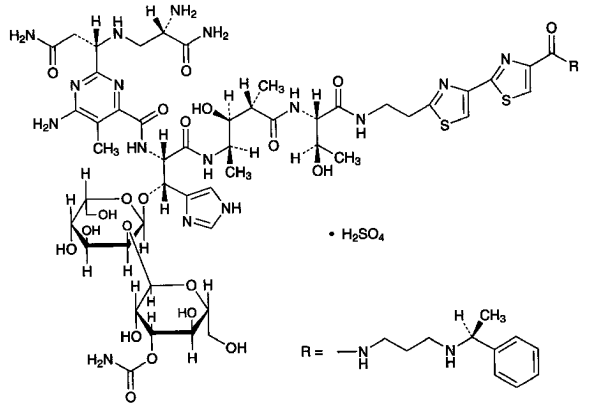
Assay Weigh accurately about 0.5 g of Pentoxyverine Citrate, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.56 mg of $C_{20}H_{31}NO_3.C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Peplomycin Sulfate

ペプロマイシン硫酸塩



$C_{61}H_{88}N_{18}O_{21}S_2.H_2SO_4$: 1571.67
N^1-{3-[(1*S*)-(1-Phenylethyl)amino]propyl}bleomycinamide monosulfate
[*70384-29-1*]

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Description Peplomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Peplomycin Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard soution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds to Qualitative Tests ⟨*1.09*⟩ (1) and (2) for sulfate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -2 – $-5°$ (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this 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 sample solution and standard solution as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Copper hollow cathode lamp.
Wavelength: 324.8 nm.

(3) Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than peplomy-

cin is not more than 7.0%.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin beginning after the peak of copper sulfate.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

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

Loss on drying ⟨*2.41*⟩ Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar media for base and seed layer, and for transferring test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iii) Liquid medium for suspending test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iv) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours. Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, incubate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate RS, equivalent to about 20 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. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly 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 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly 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 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Peplomycin Sulfate for Injection

注射用ペプロマイシン硫酸塩

Peplomycin Sulfate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Method of preparation Prepare as directed under Injections, with Peplomycin Sulfate.

Description Peplomycin Sulfate for Injection occurs as white light masses or powder.

Identification Take an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, and dissolve in 15 μL of Copper (II) sulfate TS and water to make 2 mL. Apply this solution to the column (prepared by filling a 15 mm inside diameter and 15 cm long chromatography tube with 15 mL of strongly basic ion exchange resin (Cl type) for column chromatography (75 – 150 μm in particle diameter) and run off. Then wash the column using water at 2.5 mL per minute, collect about 30 mL of the effluent. Add water to the effluent to make 250 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 242 nm and 246 nm, and between 291 nm and 295 nm. Further determine the absorbances A_1 and A_2, at 243 nm and 293 nm, respectively: the ratio A_1/A_2 is 1.20 to 1.30.

Osmotic pressure ratio Being specified separately.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 50 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is 4.5 to 6.0.

Purity Clarity and color of solution—A solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is clear and colorless.

Loss on drying <*2.41*> Not more than 4.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Perform the sampling preventing from moisture absorption.

Bacterial endotoxins <*4.01*> Less than 1.5 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

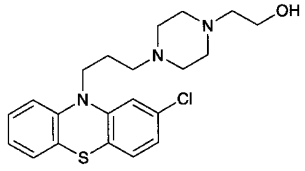
(i) Test organism, culture medium, liquid medium for suspending test organisms, preparation of seeded agar layer, preparation of cylinder-agar plate and the standard solutions —Proceed as directed in the Assay under Peplomycin Sulfate.

(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 containers of Peplomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Peplomycin Sulfate, dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8, to make exactly 100 mL. Measure exactly a suitable quantity of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Perphenazine

ペルフェナジン



$C_{21}H_{26}ClN_3OS$: 403.97

2-{4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl}ethanol

[*58-39-9*]

Perphenazine, when dried, contains not less than 98.5% of $C_{21}H_{26}ClN_3OS$.

Description Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in ethanol (95), soluble in acetic acid (100), sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification **(1)** Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt <*2.60*> between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the

same wavelengths. Separately, to 10 mL of the solution add 10 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 95 – 100°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Perphenazine 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—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 10 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 these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample 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-butanol and 1 mol/L ammonia TS (5:1) 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 sample solution is not more intense than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (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
= 20.20 mg of $C_{21}H_{26}ClN_3OS$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Perphenazine Tablets

ペルフェナジン錠

Perphenazine Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$: 403.97).

Method of preparation Prepare as directed under Tablets, with Perphenazine.

Identification (1) Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2) Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

(3) Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 Perphenazine Tablet by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about 4 μg of perphenazine ($C_{21}H_{26}ClN_3OS$) in each ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 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 the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of perphenazine ($C_{21}H_{26}ClN_3OS$)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of Perphenazine RS

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Perphenazine Tablets is not less than 70%.

Start the test with 1 tablet of Perphenazine Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 250 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$)
$= M_S \times A_T/A_S \times 1/C \times 36$

M_S: Amount (mg) of Perphenazine RS

C: Labeled amount (mg) of perphenazine ($C_{21}H_{26}ClN_3OS$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of perphenazine ($C_{21}H_{26}ClN_3OS$), add 70 mL of methanol, shake well, and add methanol to make exactly 100 mL. Filter the solution, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, and dissolve in methanol to make exactly 250 mL. Pipet 5 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 the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

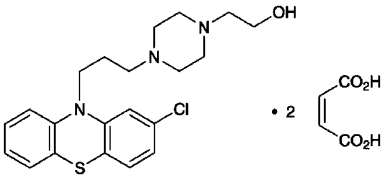
$$\text{Amount (mg) of perphenazine } (C_{21}H_{26}ClN_3OS) = M_S \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of Perphenazine RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Perphenazine Maleate

ペルフェナジンマレイン酸塩



$C_{21}H_{26}ClN_3OS.2C_4H_4O_4$: 636.11
2-{4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl}ethanol dimaleate
[*58-39-9*, Perphenazine]

Perphenazine Maleate, when dried, contains not less than 98.0% of $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$.

Description Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

Identification **(1)** Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep red-purple on warming.

(2) Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (5)]. Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <*2.60*> between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 30 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine Maleate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(5) Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts <*2.60*> between 128°C and 136°C.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of perphenazine 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) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Perphenazine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 31.81 \text{ mg of } C_{21}H_{26}ClN_3OS.2C_4H_4O_4$$

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Perphenazine Maleate Tablets

ペルフェナジンマレイン酸塩錠

Perphenazine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$: 636.11).

Method of preparation Prepare as directed under Tablets, with Perphenazine Maleate.

Identification **(1)** Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate according to the labeled amount, with 3 mL of dilute hydrochloric acid and 30 mL of water, centrifuge, filter the supernatant liquid, add 3 mL of ammonia solution (28) to the filtrate, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (4).] Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

(2) Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

(3) To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

(4) Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 50 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add water to make exactly V' mL of a solution containing about 6 μg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in each ml, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

Amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of perphenazine maleate for assay

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Perphenazine Maleate Tablets is not less than 70%.

Conduct this procedure without exposure to light. Start the test with 1 tablet of Perphenazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.5 μg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/4$

M_S: Amount (mg) of perphenazine maleate for assay
C: Labeled amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Perphenazine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

Amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of perphenazine maleate for assay

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Adsorbed Purified Pertussis Vaccine

沈降精製百日せきワクチン

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of *Bordetella pertussis* to make the antigen insoluble.

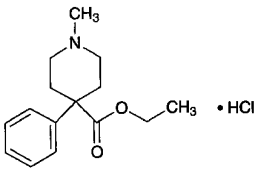
It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

Pethidine Hydrochloride

Operidine

ペチジン塩酸塩



$C_{15}H_{21}NO_2$.HCl: 283.79
Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate monohydrochloride
[*50-13-5*]

Pethidine Hydrochloride, when dried, contains not less than 98.0% of $C_{15}H_{21}NO_2$.HCl.

Description Pethidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification **(1)** Determine the absorption spectrum of a solution of Pethidine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pethidine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*> 187 – 189°C

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

(2) Sulfate <*1.14*>—Perform the test with 0.20 g of Pethidine Hydrochloride. 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 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than pethidine from the sample solution is not larger than the peak area of perthidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 μL of this solution is equivalent to 5 to 15% of that from 20 μL of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 28.38 mg of $C_{15}H_{21}NO_2.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pethidine Hydrochloride Injection

Operidine Injection

ペチジン塩酸塩注射液

Pethidine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$: 283.79).

Method of preparation Prepare as directed under Injections, with Pethidine Hydrochloride.

Description Pethidine Hydrochloride Injection is a clear, colorless liquid.
It is affected by light.
pH 4.0 – 6.0

Identification Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride according to the labeled amount, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins <*4.01*> Less than 6.0 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$), add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of pethidine hydrochloride for assay

Internal standard solution—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 12,500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 257 nm).
Column: A stainless steel column 4.6 mm in inside 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: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of pethidine 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, pethidine and the internal standard 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Hydrophilic Petrolatum

親水ワセリン

Method of preparation

White Beeswax	80 g
Stearyl Alcohol or Cetanol	30 g
Cholesterol	30 g
White Petrolatum	a sufficient quantity
	To make 1000 g

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

Description Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor.
When mixed with an equal volume of water, it retains the consistency of ointment.

Containers and storage Containers—Tight containers.

White Petrolatum

白色ワセリン

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

Description White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).

It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.

It becomes a clear liquid when warmed.

Melting point ⟨*2.60*⟩ 38 – 60°C (Method 3).

Purity **(1)** Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: Add 3.4 mL of water to 1.6 mL of Iron (III) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of White Petrolatum 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of White Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of White Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Yellow Petrolatum

黄色ワセリン

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

Description Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass, It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

Melting point ⟨*2.60*⟩ 38 – 60°C (Method 3).

Purity **(1)** Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Iron (III) Chloride CS add 1.2 mL of Cobalt (II) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Yellow Petrolatum 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Yellow Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of Yellow Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition ⟨2.44⟩ Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Petroleum Benzin

石油ベンジン

Petroleum Benzin is a mixture of low-boiling point hydrocarbons from petroleum.

Description Petroleum Benzin occurs as a colorless, clear, volatile liquid. It shows no fluorescence. It has a chracteristic odor.

It is miscible with ethanol (99.5) and with diethyl ether.

It is practically insoluble in water.

It is very flammable.

Specific gravity d_{20}^{20}: 0.65 – 0.71

Purity **(1)** Acid—Shake vigorously 10 mL of Petroleum Benzin with 5 mL of water for 2 minutes, and allow to stand: the separated aqueous layer does not change moistened blue litmus paper to red.

(2) Sulfur compounds and reducing substances—To 10 mL of Petroleum Benzin add 2.5 mL of ammonia-ethanol TS and 2 to 3 drops of silver nitrate TS, and warm the mixture at about 50°C for 5 minutes, protected from light: no brown color develops.

(3) Fatty oil and sulfur compounds—Drop and evaporate 10 mL of Petroleum Benzin in small portions on odorless filter paper spread on a previously warmed glass plate: no spot or no foreign odor is perceptible.

(4) Benzene—Warm 5 drops of Petroleum Benzin with 2 mL of sulfuric acid and 0.5 mL of nitric acid for about 10 minutes, allow to stand for 30 minutes, transfer the mixture to a porcelain dish, and dilute with water: no odor of nitrobenzene is perceptible.

(5) Residue on evaporation—Evaporate 140 mL of Petroleum Benzin on a water bath to dryness, and heat the residue at 105°C to constant mass: the mass is not more than 1 mg.

(6) Readily carbonizable substances—Shake vigorously 5 mL of Petroleum Benzin with 5 mL of sulfuric acid for readily carbonizable substances for 5 minutes in a Nessler tube, and allow to stand: the sulfuric acid layer has no more color than Matching Fluid A.

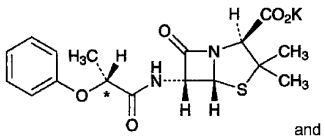
Distilling range ⟨2.57⟩ 50 – 80°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Remote from fire, and not exceeding 30°C.

Phenethicillin Potassium

フェネチシリンカリウム



and epimer at C*

$C_{17}H_{19}KN_2O_5S$: 402.51

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*RS*)-2-phenoxypropanoylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

[*132-93-4*]

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$). One unit of Phenethicillin Potassium is equivalent to 0.68 μg of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$).

Description Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Phenethicillin Potassium (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Phenethicillin Potassium responds to Qualitative Tests ⟨1.09⟩ (1) for potassium salt.

Optical rotation ⟨2.49⟩ $[\alpha]_D^{20}$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

L-α-Phenethicillin potassium Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_D and A_L, of D-α-phenethicillin and L-α-phenethicillin by the automatic integration method: $A_L/(A_D + A_L)$ is between 0.50 and 0.70.

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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 30°C.

Mobile phase: Adjust the pH of a mixture of a solution of

diammonium hydrogen phosphate (1 in 150) and acetonitrile (41:10) to 7.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of L-α-phenethicillin is about 25 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, D-α-phenethicillin and L-α-phenethicillin 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 sample solution under the above operating conditions, the relative standard deviation of the peak area of L-α-phenethicillin is not more than 2.0%.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Phenethicillin Potassium 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Phenethicillin Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than D-α-phenethicillin and L-α-phenethicillin obtained from the sample solution is not larger than 5 times the total of the peak areas of D-α-phenethicillin and L-α-phenethicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the L-α-Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-α-phenethicillin obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the L-α-Phenethicillin potassium.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Phenethicillin Potassium and dried Phenethicillin Potassium RS, equivalent to about 40,000 units, dissolve each in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 2 mL each of these solutions in 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS to them, and allow to stand for exactly 15 minutes. To them add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, and allow them to stand for exactly 15 minutes. Add 0.2 – 0.5 mL of starch TS, and titrate ⟨*2.50*⟩ with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above without allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, V_T and V_S, of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.

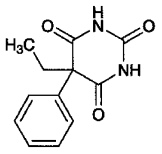
$$\text{Amount (unit) of } C_{17}H_{19}KN_2O_5S = M_S \times V_T/V_S$$

M_S: Amount (unit) of Phenethicillin Potassium RS

Containers and storage Containers—Well-closed containers.

Phenobarbital

フェノバルビタール



$C_{12}H_{12}N_2O_3$: 232.24

5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione

[*50-06-6*]

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$.

Description Phenobarbital occurs as white crystals or crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in ethanol (95) and in acetone, sparingly soluble in acetonitrile, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Identification **(1)** Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenobarbital as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 175 – 179°C

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

(2) Chloride ⟨*1.03*⟩—Dissolve 0.30 g of Phenobarbital 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) Heavy metals <1.07>—Proceed with 1.0 g of Phenobarbital 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) Phenylbarbituric acid—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

(5) Related substances—Dissolve 0.10 g of Phenobarbital in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than phenobarbital obtained from the sample solution is not larger than the peak area of phenobarbital from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 45°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

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

Time span of measurement: About 12 times as long as the retention time of phenobarbital, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of phenobarbital obtained with 10 μL of this solution is equivalent to 20 to 30% 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 phenobarbital are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenobarbital is not more than 3.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of *N*,*N*-dimethylformamide and 22 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Well-closed containers.

10% Phenobarbital Powder

Phenobarbital Powder

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

Method of preparation

Phenobarbital	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 242 nm.

(2) To 6 g of 10% Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Condense the filtrate on a water bath to about 5 mL, add about 50 mL of water, filter to collect the formed crystals, and dry them at 105°C for 2 hours. Determine the infrared absorption spectrum of the crystals as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 10% Phenobarbital Powder is not less than 80%.

Start the test with an accurately weighted about 0.3 g of 10% Phenobarbital Powder, withdraw not less than 20 mL of the medium at the specified minute 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 5 mL of the subsequent filtrate, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under

Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and water (2:1) as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Dissolution rate (%) with respect to the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 180$

M_S: Amount (mg) of phenobarbital for assay
M_T: Amount (g) of 10% Phenobarbital Powder
C: Labeled amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$) in 1 g

Assay Weigh accurately about 0.2 g of 10% Phenobarbital Powder, dissolve in a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$)
$= M_S \times A_T/A_S$

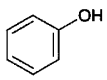
M_S: Amount (mg) of phenobarbital for assay

Containers and storage Containers—Well-closed containers.

Phenol

Carbolic Acid

フェノール



C_6H_6O: 94.11
Phenol
[*108-95-2*]

Phenol contains not less than 98.0% of C_6H_6O.

Description Phenol occurs as colorless to slightly red crystals or crystalline masses. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and soluble in water.

Phenol (10 g) is liquefied by addition of 1 mL of water.

The color changes gradually through red to dark red by light or air.

It cauterizes the skin, turning it white.

Congealing point: about 40°C

Identification **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Purity **(1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
$= 1.569$ mg of C_6H_6O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Phenol for Disinfection

Carbolic Acid for Disinfection

消毒用フェノール

Phenol for Disinfection contains not less than 95.0% of phenol (C_6H_6O: 94.11).

Description Phenol for Disinfection occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and freely soluble in water.

Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water.

It cauterizes the skin, turning it white.

Congealing point: about 30°C.

Identification **(1)** To 10 mL of a solution of Phenol for Disinfection (1 in 100) add 1 drop of iron (III) chloride TS: a blue-purple color is produced.

(2) To 5 mL of a solution of Phenol for Disinfection (1 in 10,000) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Phenol for Disinfection in 15 mL of water: the solution is clear.

(2) Residue on evaporation—Weigh accurately about 5 g

of Phenol for Disinfection, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.10% of the mass of the sample.

Assay Dissolve about 1 g of Phenol for Disinfection, accurately weighed, in water to make exactly 1000 mL. Pipet 25 mL of the solution into an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper immediately, shake well, and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Liquefied Phenol

Liquefied Carbolic Acid

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of 10% of Water, Purified Water or Purified Water in Containers.

It contains not less than 88.0% of phenol (C_6H_6O: 94.11)

Description Liquefied Phenol is a colorless or slightly reddish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with glycerin.

A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

The color changes gradually to dark red on exposure to light or air.

It cauterizes the skin, turning it white.

Specific gravity d_{20}^{20}: about 1.065

Identification **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Liquefied Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Liquefied Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Boiling point ⟨*2.57*⟩ Not more than 182°C.

Purity **(1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Liquefied Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay Dissolve about 1.7 g of Liquefied Phenol, accurately weighed, in a water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at one stopper the flask tightly, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dental Phenol with Camphor

歯科用フェノール·カンフル

Method of preparation

Phenol	35 g
d- or *dl*-Camphor	65 g
To make	100 g

Melt Phenol by warming, add *d*-Camphor or *dl*- Camphor, and mix.

Description Dental Phenol with Camphor is a colorless or light red liquid. It has a characteristic odor.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Phenol and Zinc Oxide Liniment

フェノール·亜鉛華リニメント

Method of preparation

Liquefied Phenol	22 mL
Powdered Tragacanth	20 g
Carmellose Sodium	30 g
Glycerin	30 mL
Zinc Oxide	100 g
Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 g

Mix Liquefied Phenol, Glycerin and Purified Water or Purified Water in Containers, add Powdered Tragacanth in small portions by stirring, and allow the mixture to stand overnight. To the mixture add Carmellose Sodium in small portions by stirring to make a pasty mass, add Zinc Oxide in small portions, and mix. Less than 5 g of Powdered Tragacanth or Carmellose Sodium can be replaced by each other to make 50 g in total.

Description Phenol and Zinc Oxide Liniment is a white, pasty mass. It has a slight odor of phenol.

Identification **(1)** Shake well 1 g of Phenol and Zinc Oxide Liniment with 10 mL of diethyl ether, and filter. To the filtrate add 10 mL of dilute sodium hydroxide TS, shake

well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

(2) Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chloroform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots obtained from the sample solution and the standard solution show the same *Rf* value.

Containers and storage Containers—Tight containers.

Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Liquefied Phenol	22 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Mix the above ingredients.

Description Phenolated Water is a colorless, clear liquid, having the odor of phenol.

Identification **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

(2) To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Assay Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage Containers—Tight containers.

Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Phenol for Disinfection	31 g
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Mix the above ingredients.

Description Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

Identification **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

(2) Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

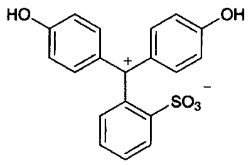
Assay Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage Containers—Tight containers.

Phenolsulfonphthalein

フェノールスルホンフタレイン



$C_{19}H_{14}O_5S$: 354.38
2-[Bis(4-hydroxyphenyl)methyliumyl]benzenesulfonate
[*143-74-8*]

Phenolsulfonphthalein, when dried, contains not less than 98.0% of $C_{19}H_{14}O_5S$.

Description Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 5 mg of Phenolsulfonphtha-

lein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well, and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.

(2) Dissolve 0.01 g of Phenolsulfonphthalein in diluted sodium carbonate TS (1 in 10) to make 200 mL. To 5 mL of this solution add diluted sodium carbonate TS (1 in 10) to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Insoluble substances—To about 1 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution of sodium hydrogen carbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, dilute with water to 100 mL, and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash with 25 mL of a solution of sodium hydrogen carbonate (1 in 100) and with five 5-mL portions of water, and dry at 105°C for 1 hour: the mass of the residue is not more than 0.2%.

(2) Related substances—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add dilute sodium hydroxide TS 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 <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of *t*-amyl alcohol, acetic acid (100) and water (4:1:1) to a distance of about 15 cm, and air-dry the plate. After allowing the plate to stand in an ammonia vapor, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250), and add water to make 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly, and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately, and shake gently for 1 minute. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 4.430 mg of $C_{19}H_{14}O_5S$

Containers and storage Containers—Well-closed containers.

Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection.

It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$: 354.38).

Method of preparation

Phenolsulfonphthalein	6 g
Sodium Chloride	9 g
Sodium Bicarbonate	1.43 g
(or Sodium Hydroxide	0.68 g)
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

Identification To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

pH <2.54> 6.0 – 7.6

Bacterial endotoxins <4.01> Less than 7.5 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Sensitivity To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

Assay Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 559 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

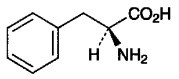
Amount (mg) of phenolsulfonphthalein ($C_{19}H_{14}O_5S$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of phenolsulfonphthalein for assay

Containers and storage Containers—Hermetic containers.

L-Phenylalanine

L-フェニルアラニン



$C_9H_{11}NO_2$: 165.19
(2*S*)-2-Amino-3-phenylpropanoic acid
[*63-91-2*]

L-Phenylalanine, when dried, contains not less than 98.5% of $C_9H_{11}NO_2$.

Description L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -33.0 – $-35.5°$ (after drying, 0.5 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Dissolve 1.0 g of L-Phenylalanine 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 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 water to make 50 mL (not more than 20 ppm).

(6) Arsenic <*1.11*>—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 <*2.03*>. Spot 5 μL each of the sample solution and 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 evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

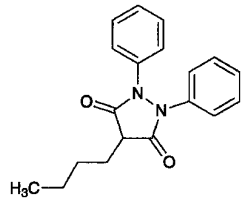
Assay Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.52 mg of $C_9H_{11}NO_2$

Containers and storage Containers—Tight containers.

Phenylbutazone

フェニルブタゾン



$C_{19}H_{20}N_2O_2$: 308.37
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione
[*50-33-9*]

Phenylbutazone, when dried, contains not less than 99.0% of $C_{19}H_{20}N_2O_2$.

Description Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

It is freely soluble in acetone, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.

(2) Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide TS, and dilute with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point ⟨*2.60*⟩ 104 – 107°C

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at 25 ± 1°C for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.05.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Phenylbutazone 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at 25 ± 1°C for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.10.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenylbutazone, previously dried, dissolve in 25 mL of acetone, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 30.84 mg of $C_{19}H_{20}N_2O_2$

Containers and storage Containers—Tight containers.

Phenylephrine Hydrochloride

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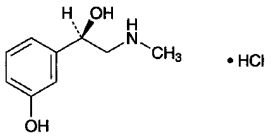


$C_9H_{13}NO_2$.HCl: 203.67
(1*R*)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride
[*61-76-7*]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_9H_{13}NO_2$.HCl.

Description Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

Identification **(1)** To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

(2) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

(3) Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts ⟨*2.60*⟩ between 170°C and 177°C.

(4) A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −42.0 – −47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 140 – 145°C

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

(2) Sulfate ⟨*1.14*⟩—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Ketone—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water, and add 2 drops of sodium pentacyanonitrosylferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

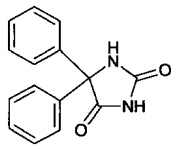
Each mL of 0.05 mol/L bromine VS
= 3.395 mg of $C_9H_{13}NO_2$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Phenytoin

Diphenylhydantoin

フェニトイン



$C_{15}H_{12}N_2O_2$: 252.27
5,5-Diphenylimidazolidine-2,4-dione
[*57-41-0*]

Phenytoin, when dried, contains not less than 99.0% of $C_{15}H_{12}N_2O_2$.

Description Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 296°C (with decomposition).

Identification **(1)** Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

(2) Boil a mixture of 0.01 g of Phenytoin, 1 mL of ammonia TS and 1 mL of water, and add dropwise 2 mL of a mixture prepared from 50 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

(3) Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

(4) Add 3 mL of chlorinated lime TS to 0.1 g of Phenytoin, shake for 5 minutes, and dissolve the oily precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point <*2.60*> is between 165°C and 169°C.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

(2) Acidity or alkalinity—Shake 2.0 g of Phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i) To 10 mL of the sample solution add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3) Chloride <*1.03*>—Dissolve 0.30 g of Phenytoin 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 from 0.60 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of acetone and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Phenytoin 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 <*2.41*> Not more than 0.5% (2 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.23 mg of $C_{15}H_{12}N_2O_2$

Containers and storage Containers—Well-closed containers.

Phenytoin Powder

Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Method of preparation Prepare as directed under Granules or Powders, with Phenytoin.

Identification Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin according to the labeled amount, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

Dissolution Being specified separately.

Assay Weigh accurately an amount of Phenytoin Powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of methanol, treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

Column: A stainless steel column 4.6 mm in inside 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 0.02 mol/L phosphate buffer solution, pH 3.5 (11:9).

Flow rate: Adjust the flow rate so that the retention time of phenytoin 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, phenytoin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Phenytoin Tablets

Diphenylhydantoin Tablets

フェニトイン錠

Phenytoin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Method of preparation Prepare as directed under Tablets, with Phenytoin.

Identification Weigh a portion of powdered Phenytoin Tablets, equivalent to about 0.3 g of Phenytoin according to the labeled amount, transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL potions of diethyl ether. Combine the extracts, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours. Proceed with the residue as directed in the Identification under Phenytoin.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Phenytoin Tablets add $3V/5$ mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 1 mg of phenytoin ($C_{15}H_{12}N_2O_2$). Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = M_S \times Q_T/Q_S \times V/25$$

M_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Phenytoin Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of a mixture of water and acetonitrile (1:1), treat with ultrasound waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 25 mL. Pipet 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

Column: A stainless steel column 4.6 mm in inside 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 0.02 mol/L phosphate buffer solution, pH 3.5 (11:9).

Flow rate: Adjust the flow rate so that the retention time of phenytoin 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, phenytoin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

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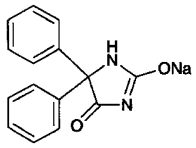

$C_{15}H_{11}N_2NaO_2$: 274.25
Monosodium 5,5-diphenyl-4-oxoimidazolidin-2-olate
[*630-93-3*]

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$), and contains not less than 92.5% and not more than 107.5% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

Method of preparation Prepare as directed under Injections.

Description Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless.

It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

It is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

Identification **(1)** With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <*1.09*> (1) for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Phenytoin Sodium for Injection 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 <*2.41*> Not more than 2.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately the content of not less than 10 preparations of Phenytoin Sodium for Injection, transfer about 0.3 g of the content, previously dried and accurately weighed, to a separator, dissolve in 50 mL of water, add 10 mL of dilute hydrochloric acid, and extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate on a water bath. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Amount (mg) of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$)
= amount (mg) of phenytoin ($C_{15}H_{12}N_2O_2$) × 1.087

Containers and storage Containers—Hermetic containers.

Phytonadione

Phytomenadione
Vitamin K_1

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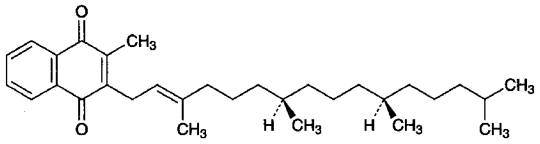


$C_{31}H_{46}O_2$: 450.70
2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]-1,4-naphthoquinone
[*84-80-0*]

Phytonadione contains not less than 97.0% and not more than 102.0% of $C_{31}H_{46}O_2$.

Description Phytonadione is a clear yellow to orange-yellow, viscous liquid.

It is miscible with isooctane.

It is soluble in ethanol (99.5), and practically insoluble in water.

It decomposes gradually and changes to a red-brown by light.

Specific gravity d_{20}^{20}: about 0.967

Identification **(1)** Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phytonadione as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.525 – 1.529

Purity **(1)** Ratio of absorbances—Determine the absorbances, A_1, A_2 and A_3, of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the ratio A_2/A_1 is between 0.69 and 0.73, and the ratio A_2/A_3 is between 0.74 and 0.78. Determine the absorbances, A_4 and A_5, of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio A_4/A_5 is between 0.28 and 0.34.

(2) Heavy metals ⟨*1.07*⟩—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed 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) Menadione—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

Isomer ratio Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of *Z*-isomer and *E*-isomer, A_{TZ} and A_{TE}: $A_{TZ}/(A_{TZ} + A_{TE})$ is between 0.05 and 0.18.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the sample solution under the above operating conditions, *Z*-isomer and *E*-isomer 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 50 μL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the peaks of *Z*-isomer and *E*-isomer is not more than 2.0%.

Assay Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione RS, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solutions add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these as the sample solution and the standard solution, respectively. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard.

$$\text{Amount (mg) of } C_{31}H_{46}O_2 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Phytonadione RS

Internal standard solution—A solution of cholesterol benzoate in the mobile phase (1 in 400).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with porous 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 *n*-amyl alcohol (4000 : 3).

Flow rate: Adjust the flow rate so that the retention time of the peak of *E*-isomer of phytonadione is about 25 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the internal standard, *Z*-isomer and *E*-isomer are eluted in this order with the resolution between the peaks of *Z*-isomer and *E*-isomer being not less than 1.5.

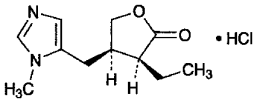
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a cold place or in containers in which air has been displaced by Nitrogen.

Pilocarpine Hydrochloride

ピロカルピン塩酸塩



$C_{11}H_{16}N_2O_2$.HCl: 244.72

(3*S*,4*R*)-3-Ethyl-4-(1-methyl-1*H*-imidazol-5-ylmethyl)-4,5-dihydrofuran-2(3*H*)-one monohydrochloride

[*54-71-7*]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of $C_{11}H_{16}N_2O_2$.HCl.

Description Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in ethanol (95), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.

It is hygroscopic.

It is affected by light.

Identification **(1)** Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and

shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

(2) To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

Melting point ⟨*2.60*⟩ 200 – 203°C

Purity **(1)** Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

(3) Related substances—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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 TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Readily carbonizable substances ⟨*1.15*⟩—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

Loss on drying ⟨*2.41*⟩ Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (0.1 g).

Assay Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

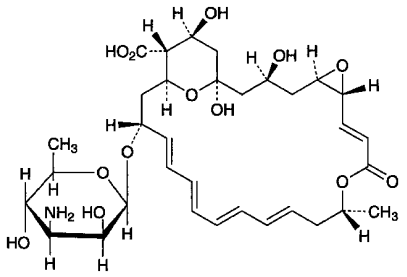
Each mL of 0.1 mol/L perchloric acid VS
= 24.47 mg of $C_{11}H_{16}N_2O_2.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pimaricin

Natamycin

ピマリシン



$C_{33}H_{47}NO_{13}$: 665.73
(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.0^{5,7}]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid
[*7681-93-8*]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

It contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin ($C_{33}H_{47}NO_{13}$).

Description Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

Identification **(1)** To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Pimaricin 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).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 303 nm).

Column: A stainless steel column 3.9 mm in inside 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 40°C.

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

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

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

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

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

Water ⟨*2.48*⟩ Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pimaricin and Pimaricin RS, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL each of these solutions, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances at 295.5 nm, A_{T1} and A_{S1}, at 303 nm, A_{T2} and A_{S2}, and at 311 nm, A_{T3} and A_{S3}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Amount [}\mu\text{g (potency)] of } C_{33}H_{47}NO_{13} = M_S \times \frac{A_{T2} - \dfrac{A_{T1} + A_{T3}}{2}}{A_{S2} - \dfrac{A_{S1} + A_{S3}}{2}} \times 1000$$

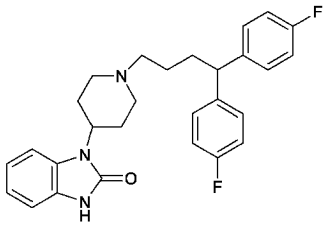
M_S: Amount [mg (potency)] of Pimaricin RS

Containers and storage Containers—Tight containers.

Storage—Light resistant.

Pimozide

ピモジド



$C_{28}H_{29}F_2N_3O$: 461.55

1-{1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl}-1,3-dihydro-2*H*-benzoimidazol-2-one

[*2062-78-4*]

Pimozide contains not less than 98.5% and not more than 101.0% of $C_{28}H_{29}F_2N_3O$.

Description Pimozide occurs as a white to pale yellowish white powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Pimozide in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pimozide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 216 – 220°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pimozide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution by using 5 mL of sulfuric acid (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Pimozide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Pimozide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the peak of pimozide from the sample solution is not lager than the peak area of pimozide from the standard solution, and the total area of the peaks other than the peak of pimozide from the sample solution is not larger than 1.5 times of the peak area of pimozide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm 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 A: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	80 → 70	20 → 30
10 – 15	70	30

Flow rate: 2.0 mL per minute.

Time span of measurement: 1.5 times as long as the retention time of pimozide.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozide obtained from 10 μL of this solution is equivalent to 8 to 12% of that of pimozide from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Pimozide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozide 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pimozide is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

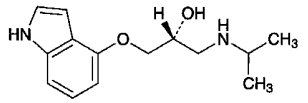
Assay Weigh accurately about 70 mg of Pimozide, previously dried, dissolve in 25 mL of acetic acid for nonaqueous titration, and titrate ⟨*2.50*⟩ with 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 9.231 mg of $C_{28}H_{29}F_2N_3O$

Containers and storage Containers—Well-closed containers.

Pindolol

ピンドロール



and enantiomer

$C_{14}H_{20}N_2O_2$: 248.32

(2*RS*)-1-(1*H*-Indol-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol

[*13523-86-9*]

Pindolol, when dried, contains not less than 98.5% of $C_{14}H_{20}N_2O_2$.

Description Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in dilute sulfuric acid and in acetic acid (100).

Identification **(1)** To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

(2) Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Pindolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pindolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨*2.24*⟩ $E_{1\,cm}^{1\%}$ (264 nm): 333 – 350 (10 mg, methanol, 500 mL).

Melting point ⟨*2.60*⟩ 169 – 173°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 4 mL of Matching Fluid A, add exactly 6 mL of water, and mix.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Pindolol 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Pindolol according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pindolol in 10 mL of methanol, and use this solution as the sample solu-

tion. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 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 <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

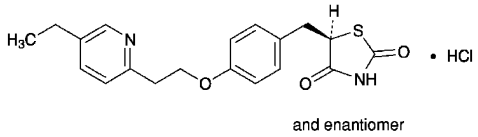
Assay Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 24.83 mg of $C_{14}H_{20}N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pioglitazone Hydrochloride

ピオグリタゾン塩酸塩



and enantiomer

$C_{19}H_{20}N_2O_3S.HCl$: 392.90
(5*RS*)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}thiazolidine-2,4-dione monohydrochloride
[*112529-15-4*]

Pioglitazone Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{19}H_{20}N_2O_3S$.HCl, calculated on the anhydrous basis.

Description Pioglitazone Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide and in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Pioglitazone Hydrochloride in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pioglitazone Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pioglitazone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pioglitazone Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Pioglitazone Hydrochloride in 1 mL of nitric acid, and add 4 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pioglitazone Hydrochloride according to Method 4, and perform the test. After incineration, use 3 mL of hydrobromic acid instead of 3 mL of hydrochloric acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Pioglitazone Hydrochloride in 20 mL of methanol, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention times of about 0.7, about 1.4 and about 3.0 with respect to pioglitazone from the sample solution, is not larger than 2/5 times the peak area of pioglitazone from the standard solution, and the area of each peak other than the peak of pioglitazone and other than those mentioned above is smaller than 1/5 times the peak area of pioglitazone from the standard solution. Furthermore, the total area of the peaks other than the peak of pioglitazone is not larger than the peak area of pioglitazone 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.

Time span of measurement: About 4 times as long as the retention time of pioglitazone, beginning after the solvent peak.

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 pioglitazone obtained from 40 μL of this solution is equivalent to 7 to 13% of that of pioglitazone from 40 μL of the standard solution.

System performance: Dissolve 50 mg of Pioglitazone Hydrochloride in 10 mL of a solution of benzophenone in methanol (1 in 750), and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 40 μL of this solution under the above operating conditions, pioglitazone and benzophenone 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 40 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of pioglitazone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ Not more than 0.2% (0.5 g, coulometric titration). For anolyte solution, use anolyte solution for water determination A.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Pioglitazone Hydrochloride and Pioglitazone Hydrochloride RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Pioglitazone Hydrochloride), add exactly 10 mL of the internal standard solution and methanol to make 100 mL. Pipet 2 mL each of these solutions, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside 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 ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone 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, pioglitazone 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pioglitazone Hydrochloride Tablets

ピオグリタゾン塩酸塩錠

Pioglitazone Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl: 392.90).

Method of preparation Prepare as directed under Tablets, with Pioglitazone Hydrochloride.

Identification To an amount of powdered Pioglitazone Hydrochloride Tablets, equivalent to 2.8 mg of Pioglitazone Hydrochloride according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 267 nm and 271 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Pioglitazone Hydrochloride Tablets with 10 mL of 0.1 mol/L hydrochloric acid TS, add 70 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and centrifuge. Take exactly V mL of the supernatant liquid, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly V' mL so that each mL contains about 26 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) as the blank.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 2/25$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, which is prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 45 minutes of Pioglitazone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride Tablets, withdraw 10 mL of the medium at the specified minute after starting 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 medium to make exactly V' mL so that each mL contains about 18 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis
C Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Pioglitazone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), add 45 mL of methanol and exactly 5 mL of the internal standard solution, agitate with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 45 mL of methanol, and add exactly 5 mL of the internal standard solution. Pipet 2 mL of this solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside 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 ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone 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, pioglitazone 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pipemidic Acid Hydrate

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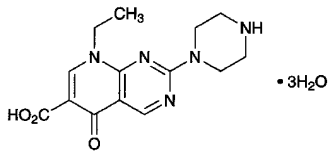

$C_{14}H_{17}N_5O_3.3H_2O$: 357.36
8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid trihydrate
[*51940-44-4*, anhydride]

Pipemidic Acid Hydrate contains not less than 98.5% and not more than 101.0% of pipemidic acid ($C_{14}H_{17}N_5O_3$: 303.32), calculated on the anhydrous basis.

Description Pipemidic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5), and practically insoluble in methanol.

It dissolves in sodium hydroxide TS.

It is gradually colored on exposure to light.

Melting point: about 250°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pipemidic Acid Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride <1.03>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute nitric acid, shake well, and filter through a glass filter (G3). 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 as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <*1.14*>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute hydrochloric acid, shake well, and filter through a glass filter (G3). 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 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Pipemidic 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).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Pipemidic Acid Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetic acid (100) (1 in 20) 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 <*2.03*>. Spot 5 μL each of the sample 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, methanol, formic acid and triethylamine (25:15:5:1) 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 sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 14.5 – 16.0% (20 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piperacillin Hydrate

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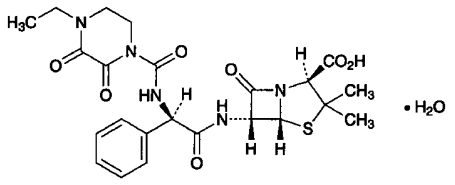

$C_{23}H_{27}N_5O_7S.H_2O$: 535.57
(2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate
[*66258-76-2*]

Piperacillin Hydrate contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Hydrate is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description Piperacillin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5) and in dimethylsulfoxide, and very slightly soluble in water.

Identification **(1)** Determine the infrared absorption spectrum of Piperacillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Piperacillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Piperacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metal <*1.07*>—Proceed with 2.0 g of Piperacillin 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 1—Conduct this procedure rapidly after the preparation of the sample solution and standard solution. Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the 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 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and

determine each peak area by the automatic integration method: the total area of the peaks, having the relative retention time of about 0.38 and about 0.50 with respect to piperacillin, obtained from the sample solution is not larger than 2 times the peak area of piperacillin from the standard solution (2), the total area of the peaks, having the relative retention time of about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (2), and the area of the peak other than piperacillin and other than the peaks having the relative retention time of about 0.38, about 0.50, about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution, is not larger than the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than piperacillin obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (1).

Operating conditions—

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

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

System suitability—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

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 and the symmetry factor of the peak of piperacillin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 3.0%.

(3) Related substances 2—Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the 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 20 μL each of the sample solution, and the standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> 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 6.6 with respect to piperacillin, obtained from the sample solution is not larger than 3 times the peak area of piperacillin from the standard solution (2), and the area of the peaks other than the peak of piperacillin and the peak having the relative retention time of about 6.6 with respect to piperacillin from the sample solution are not larger than 1.4 times the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than the peak of piperacillin from the sample solution is not larger than the area of the peak of piperacillin from the standard solution (1). For these calculations, use the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, after multiplying by the relative response factor, 2.0.

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 300 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 1.2 minutes.

Time span of measurement: About 8 times as long as the retention time of piperacillin, beginning after the piperacillin peak.

System suitability—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

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 and the symmetry factor of the peak of piperacillin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 4.0%.

(4) Residual solvents <*2.46*>—Transfer exactly 10 mg of Piperacillin Hydrate to an about 3 mL-vial, add exactly 1 mL of saturated sodium hydrogen carbonate solution to dissolve and stop the vial tightly. After heating this at 90°C for 10 minutes, use the gas inside the container as the sample gas. Separately, measure exactly 1 mL of ethyl acetate, dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 20 mL. Pipet 2 μL of this solution in an about 3-mL vial containing exactly 1 mL of saturated sodium hydrogen carbonate solution, and stop the vial tightly. Run the procedure similarly to the sample, and use the gas as the standard gas. Perform the test with exactly 0.5 mL each of the sample gas and standard gas as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area of ethyl acetate by the automatic integration method: the peak area of ethyl acetate obtained from the sample gas is not larger than that from the standard gas.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous stylene-divinyl benzene copolymer for gas chromatography (average pore diameter of 0.0085 μm, 300 – 400 m^2/g) with the particle size of 125 to 150 μm.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 4 minutes.

System suitability—

System performance: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL each of ethyl acetate solution (1 in 400) and acetone solution (1 in 400), and stop the vial tightly. When the procedure

is run under the above operating conditions, acetone and ethyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL of ethyl acetate solution (1 in 400), stop the vial tightly, and perform the test under the above operating conditions. When the procedure is repeated 6 times, the relative standard deviation of the peak area of ethyl acetate is not more than 10%.

Water ⟨*2.48*⟩ Not less than 3.2% and not more than 3.8% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.07 EU/mg (potency).

Assay Weigh accurately an amount of Piperacillin Hydrate and Piperacillin RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, H_T and H_S, of the peak height of piperacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of piperacillin } (C_{23}H_{27}N_5O_7S) = M_S \times H_T/H_S \times 1000$$

M_S: Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 210 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin 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 internal standard and piperacillin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Piperacillin Sodium

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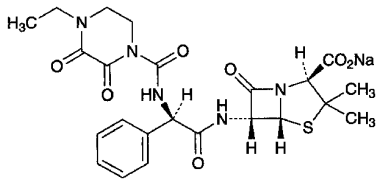


$C_{23}H_{26}N_5NaO_7S$: 539.54

Monosodium (2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

[*59703-84-3*]

Piperacillin Sodium contains not less than 863 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description Piperacillin Sodium occurs as a white powder or mass.

It is very soluble in water, freely soluble in methanol and in ethanol (95), and practically insoluble in acetonitrile.

Identification **(1)** Determine the infrared absorption spectrum of Piperacillin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Piperacillin Sodium responds to Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +175 – +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Piperacillin Sodium in 4 mL of water: the pH of the solution is between 5.0 and 7.0.

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Piperacillin 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 ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.1 g of Piperacillin Sodium in 50 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 times that of piperacillin from the standard solu-

tion, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compounds 1 and related compound 2 are used after multiplying by their relative response factors, 1.39, 1.32 and 1.11, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogenphosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogenphosphate (25:24:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 7	100	0
7 – 13	100 → 83	0 → 17
13 – 41	83	17
41 – 56	83 → 20	17 → 80
56 – 60	20	80

Flow rate: 1.0 mL per minute. The retention time of piperacillin is about 33 minutes.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

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 piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of piperacillin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase 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 standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of piperacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of piperacillin } (C_{23}H_{27}N_5O_7S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin 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 internal standard and piperacillin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Piperacillin Sodium for Injection

注射用ピペラシリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Method of preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection is a white powder or masses.

Identification Proceed as directed in the Identification under Piperacillin Sodium.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium according to the la-

beled amount, in 4 mL of water is 5.0 – 7.0.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

(2) Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

Water ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.04 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium, dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Piperacillin RS, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

$$\text{Amount [mg (potency)] of piperacillin } (C_{23}H_{27}N_5O_7S) = M_S \times Q_T/Q_S$$

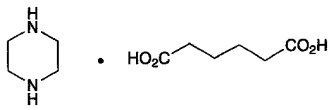
M_S: Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Piperazine Adipate

ピペラジンアジピン酸塩



$C_4H_{10}N_2.C_6H_{10}O_4$: 232.28
Piperazine hexanedioate
[*142-88-1*]

Piperazine Adipate, when dried, contains not less than 98.5% of $C_4H_{10}N_2.C_6H_{10}O_4$.

Description Piperazine Adipate occurs as a white, crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in water and in acetic acid (100), and practically insoluble in ethanol (95), in acetone and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification **(1)** Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point ⟨*2.60*⟩ is between 152°C and 155°C.

(2) To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Piperazine Adipate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH ⟨*2.54*⟩ The pH of a solution of Piperazine Adipate (1 in 20) is between 5.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Piperazine Adipate 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).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 11.61 \text{ mg of } C_4H_{10}N_2.C_6H_{10}O_4$$

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Hydrate

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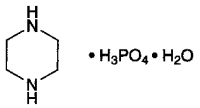


$C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15
Piperazine monophosphate monohydrate
[*18534-18-4*]

Piperazine Phosphate Hydrate contains not less

than 98.5% of piperazine phosphate ($C_4H_{10}N_2.H_3PO_4$: 184.13), calculated on the anhydrous basis.

Description Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in formic acid, sparingly soluble in water, very slightly soluble in acetic acid (100), and practically insoluble in methanol, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

Melting point: about 222°C (with decomposition).

Identification **(1)** To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS:a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Piperazine Phosphate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <*1.09*> (1) and (3) for phosphate.

pH <*2.54*> Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

Purity **(1)** Chloride <*1.03*>—To 0.5 g of Piperazine Phosphate Hydrate 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Heavy metals <*1.07*>—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add 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 (not more than 10 ppm).

(3) Arsenic <*1.11*>—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Piperazine Phosphate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28), acetone and ethanol (99.5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 8.0 – 9.5% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 mL of acetic acid (100), and titrate <*2.50*> 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
= 9.207 mg of $C_4H_{10}N_2.H_3PO_4$

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Tablets

ピペラジンリン酸塩錠

Piperazine Phosphate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15).

Method of preparation Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

Identification Take a quantity of Piperazine Phosphate Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate according to the labeled amount, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

Disintegration <*6.09*> It meets the requirement. The time limit of the test is 10 minutes.

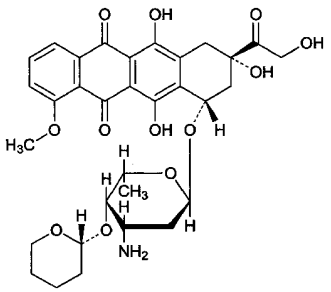
Assay Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$). Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, add 50 mL of acetic acid (100), and titrate <*2.50*> 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
= 10.11 mg of $C_4H_{10}N_2.H_3PO_4.H_2O$

Containers and storage Containers—Tight containers.

Pirarubicin

ピラルビシン



$C_{32}H_{37}NO_{12}$: 627.64
(2*S*,4*S*)-4-{3-Amino-2,3,6-trideoxy-4-*O*-[(2*R*)-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyloxy}-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione
[*72496-41-4*]

Pirarubicin is a derivative of daunorubicin.

It contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin ($C_{32}H_{37}NO_{12}$).

Description Pirarubicin occurs as a red-orange crystalline powder.

It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Pirarubicin and Pirarubicin RS in 5 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same *R*f value.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +195 – +215° (10 mg, chloroform, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Pirarubicin 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 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not larger than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not larger than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

Operating conditions—

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

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <*2.48*> Not more than 2.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pirarubicin and Pirarubicin RS, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pirarubicin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{32}H_{37}NO_{12} = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Pirarubicin RS

Internal standard solution—A solution of 2-naphthol in the mobile phase (1 in 1000).

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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 0.05 mol/L ammonium formate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

System suitability—

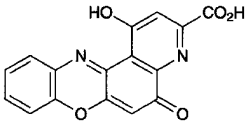
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Pirenoxine

ピレノキシン



$C_{16}H_8N_2O_5$: 308.25

1-Hydroxy-5-oxo-5*H*-pyrido[3,2-*a*]phenoxazine-3-carboxylic acid

[*1043-21-6*]

Pirenoxine, when dried, contains not less than 98.0% of $C_{16}H_8N_2O_5$.

Description Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95), in tetrahydrofuran and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification **(1)** Dissolve 2 mg of Pirenoxine in 10 mL of phosphate buffer solution, pH 6.5, add 5 mL of a solution of L-ascorbic acid (1 in 50), and shake vigorously: a dark purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Pirenoxine in phosphate buffer solution, pH 6.5 (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Pirenoxine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pirenoxine 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 Pirenoxine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 pirenoxine is not larger than the peak area of pirenoxine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside 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°C.

Mobile phase: Dissolve 1.39 g of tetra *n*-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

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

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

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 μL of this solution is equivalent to 5 to 8% of that of pirenoxine obtained from 5 μL of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, pirenoxine and methyl parahydroxybenzoate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenoxine is not more than 1.0%.

Loss on drying <*2.41*> Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

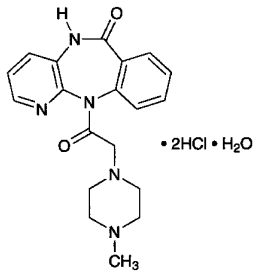
Assay Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate <*2.50*> immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS
= 6.165 mg of $C_{16}H_8N_2O_5$

Containers and storage Containers—Tight containers.

Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物



$C_{19}H_{21}N_5O_2.2HCl.H_2O$: 442.34

11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride monohydrate

[*29868-97-1*, anhydride]

Pirenzepine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of pirenzepine hydrochloride ($C_{19}H_{21}N_5O_2.2HCl$: 424.32), calculated on the anhydrous basis.

Description Pirenzepine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

It is freely soluble in water and in formic acid, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

The pH of a solution by dissolving 1 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is between 1.0 and 2.0.

Melting point: about 245°C (with decomposition).

It is gradually colored by light.

Identification **(1)** Determine the absorption spectrum of a solution of Pirenzepine Hydrochloride Hydrate (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pirenzepine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pirenzepine Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is clear and not more color than that of the following control solution.

Control solution: To 1.2 mL of Matching fluid for color F add 8.8 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Pirenzepine 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).

(3) Related substances—Dissolve 0.3 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water. To 1 mL of this solution add 5 mL of methanol and the mobile phase A to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add 5 mL of methanol and the mobile phase A to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pirenzepine is not larger than 3/10 times the peak area of pirenzepine from the standard solution, and the total area of the peaks other than pirenzepine is not larger than 3/5 times the peak area of pirenzepine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 2 g of sodium lauryl sulfate in 900 mL of water, adjust the pH to 3.2 with acetic acid (100), and add water to make 1000 mL.

Mobile phase B: Methanol.

Mobile phase C: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 15	55 → 25	30	15 → 45
15 –	25	30	45

Flow rate: Adjust the flow rate so that the retention time of pirenzepine is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of pirenzepine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Confirm that the peak area of pirenzepine obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 0.1 g of phenylpiperazine hydrochloride in 10 mL of methanol. Mix 1 mL of this solution and 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pirenzepine and phenylpiperazine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenzepine is not more than 2.0%.

Water <*2.48*> Not less than 3.5% and not more than 5.0%

(0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pirenzepine Hydrochloride Hydrate, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

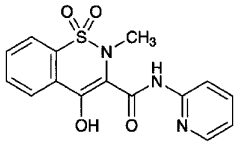
Each mL of 0.1 mol/L perchloric acid VS
= 14.14 mg of $C_{19}H_{21}N_5O_2.2HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piroxicam

ピロキシカム



$C_{15}H_{13}N_3O_4S$: 331.35
4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide
[*36322-90-4*]

Piroxicam contains not less than 98.5% and not more than 101.0% of $C_{15}H_{13}N_3O_4S$, calculated on the dried basis.

Description Piroxicam occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Piroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Piroxicam 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 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than piroxicam obtained with the sample solution is not larger than the peak area of piroxicam with the standard solution, and the total area of the peaks other than piroxicam is not larger than 2 times the peak area of piroxicam with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile for liquid chromatography (3:2).

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

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

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 μL of this solution is equivalent to 17.5 to 32.5% 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 piroxicam are not less than 6000 and not more than 1.5, respectively.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

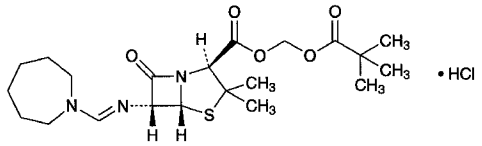
Assay Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.14 mg of $C_{15}H_{13}N_3O_4S$

Containers and storage Containers—Tight containers.

Pivmecillinam Hydrochloride

ピブメシリナム塩酸塩



$C_{21}H_{33}N_3O_5S.HCl$: 476.03

2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[(azepan-1-ylmethylene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [*32887-03-9*]

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) and not more than 710 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

Description Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

Identification **(1)** Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate 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 amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride RS in 4.0 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

Water <*2.48*> Not more than 1.0% (0.25 g, coulometric titration).

Assay Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pivmecillinam to that of the internal standard.

Amount [μg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), 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 pivmecillinam 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, pivmecillinam 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pivmecillinam Hydrochloride Tablets

ピブメシリナム塩酸塩錠

Pivmecillinam Hydrochloride Tablets contains not less than 93.0% and not more than 107.0% of the labeled potency of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

Method of preparation Prepare as directed under Tablets, with Pivmecillinam Hydrochloride.

Identification Powder Pivmecillinam Hydrochloride Tablets, dissolve a portion of the powder, equivalent to 35 mg (potency) of Pivmecillinam Hydrochloride according to the labeled amount, in 4 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately dissolve 25 mg of Pivmecillinam Hydrochloride RS in 2 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and immediately develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

Water <*2.48*> Not more than 3.0% (1 g of powdered Pivmecillinam Hydrochloride Tablets, volumetric titration, direct titration).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pivmecillinam Hydrochloride Tablets add 40 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 50 mL. Pipet V mL, equivalent to about 10 mg (potency) of Pivmecillinam Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, 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 sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
$= M_S \times Q_T/Q_S \times 25/V$

M_S: Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Disintegration <*6.09*> Perform the test using the disk: it meets the requirement.

Assay Weigh accurately the mass of not less than 20 Pivmecillinam Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Pivmecillinam Hydrochloride, add 50 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, 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 sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Containers and storage Containers—Tight containers.

Live Oral Poliomyelitis Vaccine

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III.

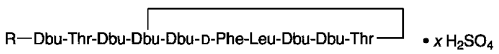
Monovalent or bivalent product may be prepared, if necessary.

Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

Description Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

Polymixin B Sulfate

ポリミキシン B 硫酸塩



Polymyxin B_1 : R = 6-Methyloctanoic acid
Dbu = L-α, γ-Diaminobutyric acid

Polymyxin B_2 : R = 6-Methylheptanoic acid
Dbu = L-α, γ-Diaminobutyric acid

Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B

Sulfate is expressed as mass unit of polymixin B ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}$). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}.1\text{-}2H_2SO_4$).

Description Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate RS separately into two glass stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and standard solution (1). Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine separately in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4) and (5), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 3 μL each of the sample solution, the standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography, and expose the plate to a saturated vapor of the developing solvent for 15 hours. Develop the plate with a mixture of phenol and water (3:1) to a distance of about 13 cm while without exposure to light, and dry the plate at 110°C for 5 minutes. Spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110°C for 5 minutes: *R*f value of each spot obtained from the sample solution is the same with *R*f value of the corresponding spots from the standard solution (1). Each of the spots from the sample solution appears at the position corresponding to each of the spots from the standard solutions (2), (3) and (4), but not appears at the position corresponding to the spot from the standard solution (5).

(3) A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -78 – $-90°$ (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

Phenylalanine Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine absorbances, A_1, A_2, A_3, A_4 and A_5, of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine calculated on the dried basis is not less than 9.0% and not more than 12.0%.

Amount (%) of phenylalanine
$= (A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5)/M_T \times 9.4787$

M_T: Amount (g) of the sample, calculated on the dried basis

Purity Heavy metals <*1.07*>—Proceed with 1.0 g of Polymixin B 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).

Loss on drying <*2.41*> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.75% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Agar media for seed and base layer

Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH <*2.54*> of the solution so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate RS, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Polyoxyl 40 Stearate

ステアリン酸ポリオキシル **40**

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula $H(OCH_2CH_2)_nOCOC_{17}H_{35}$, in which n is approximately 40.

Description Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

Congealing point <*2.42*> 39.0 – 44.0°C

Congealing point of the fatty acid <*1.13*> Not below 53°C.

Acid value <*1.13*> Not more than 1.

Saponification value <*1.13*> 25 – 35

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Polyoxyl 40 Stearate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Polyoxyl 40 Stearate 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 <*1.11*>—Prepare the test solution with 0.67 g of Polyoxyl 40 Stearate, according to Method 3, and perform the test (not more than 3 ppm).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Polysorbate 80

ポリソルベート **80**

Polysorbate 80 is a polyoxyethylene ether of anhydrous sorbitol, partially esterified with oleic acid.

Description Polysorbate 80 is a colorless or orange-yellow, viscous liquid, having a faint, characteristic odor and a warm, slightly bitter taste.

It is miscible with methanol, with ethanol (95), with warm ethanol (95), with pyridine and with chloroform.

It is freely soluble in water and slightly soluble in diethyl ether.

The pH of a solution of Polysorbate 80 (1 in 20) is between 5.5 and 7.5.

Identification **(1)** To 5 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of sodium hydroxide TS, boil for 5 minutes, cool, and acidify with dilute hydrochloric acid: the solution is opalescent.

(2) To 5 mL of a solution of polysorbate 80 (1 in 20) add 2 to 3 drops of bromine TS: the color of the test solution is discharged.

(3) Mix 6 mL of Polysorbate 80 with 4 mL of water at an ordinary, or lower than ordinary, temperature: a jelly-like mass is produced.

(4) To 10 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, shake well, add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

Viscosity <*2.53*> 345 – 445 mm^2/s (Method 1, 25°C).

Specific gravity <*1.13*> d_{20}^{20}: 1.065 – 1.095

Acid value <*1.13*> Not more than 2.0.

Saponification value <*1.13*> 45 – 55

Iodine value <*1.13*> 19 – 24 Use chloroform instead of cyclohexane, and titrate <*2.50*> without using an indicator, until the yellow color of iodine disappears.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Polysorbate 80 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 <*1.11*>—Prepare the test solution with 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 3.0% (1 g, volumetric titration, back titration).

Residue on ignition <*2.44*> Not more than 0.1% (2 g).

Containers and storage Containers—Tight containers.

Potash Soap

カリ石ケン

Potash Soap contains not less than 40.0% as fatty acids.

Method of preparation

Fixed oil	470 mL
Potassium Hydroxide	a sufficient quantity
Water, Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 g

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water, Purified Water or Purified Water in Containers, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water, Purified Water or Purified Water in Containers to make 1000 g.

Description Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.

It is freely soluble in water and in ethanol (95).

Purity Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

Assay Weigh accurately about 5 g of Potash Soap, dissolve in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Transfer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

Containers and storage Containers—Tight containers.

Potassium Bromide

臭化カリウム

KBr: 119.00

Potassium Bromide, when dried, contains not less than 99.0% of KBr.

Description Potassium Bromide occurs as colorless or

white crystals, granules or crystalline powder. It is odorless.

It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

Identification A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests <*1.09*> for potassium salt and for bromide.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.

(3) Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

(4) Sulfate <*1.14*>—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Bromide 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).

(8) Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, 110°C, 4 hours).

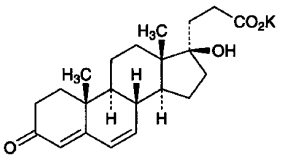
Assay Weigh accurately about 0.4 g of Potassium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS, and titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 11.90 mg of KBr

Containers and storage Containers—Tight containers.

Potassium Canrenoate

カンレノ酸カリウム



$C_{22}H_{29}KO_4$: 396.56
Monopotassium 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate
[*2181-04-6*]

Potassium Canrenoate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{22}H_{29}KO_4$.

Description Potassium Canrenoate occurs as a pale yellowish white to pale yellow-brown, crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

Identification **(1)** Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

(2) Determine the absorption spectrum of a solution of Potassium Canrenoate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Potassium Canrenoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests <*1.09*> (1) for potassium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -71 – $-76°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear, and shows a pale yellow to light yellow color.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Canrenoate 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 <*1.11*>—Prepare the test solution with 1.0 g of Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

(4) Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a

temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it is not more than 0.67.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.2 g of Potassium Canrenoate, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Use a solution of saturated potassium chloride-acetic acid (100) as the internal liquid.). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.66 mg of $C_{22}H_{29}KO_4$

Containers and storage Containers—Tight containers.

Potassium Carbonate

炭酸カリウム

K_2CO_3: 138.21

Potassium Carbonate, when dried, contains not less than 99.0% of K_2CO_3.

Description Potassium Carbonate occurs as white granules or powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (95).

A solution of Potassium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests <*1.09*> for potassium salt and for carbonate.

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

(2) Heavy metals <*1.07*>—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution to dryness, and dilute with water to 50 mL (not more than 20 ppm).

(3) Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test <*1.04*> (1): no persisting yellow color is produced.

(4) Arsenic <*1.11*>—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

Loss on drying <*2.41*> Not more than 1.0% (3 g, 180°C, 4 hours).

Assay Dissolve about 1.5 g of Potassium Carbonate, previously dried and accurately weighed, in 25 mL of water, titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate <*2.50*> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 69.11 mg of K_2CO_3

Containers and storage Containers—Tight containers.

Potassium Chloride

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99% of KCl.

Description Potassium Chloride occurs as colorless or white crystals or crystalline powder. It is odorless, and has a saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Potassium Chloride (1 in 10) is neutral.

Identification A solution of Potassium Chloride (1 in 50) responds to Qualitative Tests <*1.09*> for potassium salt and for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Bromide—Dissolve 1.0 g of Potassium Chloride in water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluensulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

(4) Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.

(5) Heavy metals <*1.07*>—Proceed with 4.0 g of Potassium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(6) Calcium and magnesium—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS, and then allow to stand for 5 minutes: no turbidity is produced.

(7) Sodium—Dissolve 1.0 g of Potassium Chloride in 20 mL of water, and perform the Flame Coloration Test <*1.04*> (1): no persistent, yellow color develops.

(8) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 130°C, 2 hours).

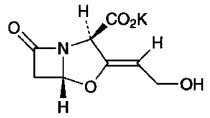
Assay Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS = 7.455 mg of KCl

Containers and storage Containers—Tight containers.

Potassium Clavulanate

クラブラン酸カリウム



$C_8H_8KNO_5$: 237.25
Monopotassium (2*R*,5*R*)-3-[(1*Z*)-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*61177-45-5*]

Potassium Clavulanate is the potassium salt of a substance having β-lactamase inhibiting activity produced by the growth of *Streptomyces clavuligerus*.

It contains not less than 810 μg (potency) and not more than 860 μg (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavularic acid ($C_8H_9NO_5$: 199.16).

Description Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Potassium Clavulanate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Potassium Clavulanate responds to Qualitative Tests <*1.09*> (1) for potassium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +53 – +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Clavulanate 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 <*1.11*>—Prepare the test solution with 1.0 g of Potassium Clavulanate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not larger than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not larger than 2 times of 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 4.6 mm in inside diameter and 10 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: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A and methanol (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (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 per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

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: Dissolve 10 mg each of Potassium Clavulanate and Amoxycillin in 100 mL of the mobile phase A. When the procedure is run with 20 μL of this solution under the above operating conditions, clavulanic acid and amoxycillin are eluted in this order with the resolution between these peaks being not less than 8 and the number of theoretical plates of the peak of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.5% (5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Potassium Clavulanate and Lithium Clavulanate RS, equivalent to about 12.5 mg (potency), dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clavularic acid to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of clavularic acid } (C_8H_9NO_5) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Lithium Clavulanate RS

Internal standard solution—Dissolve 0.3 g of sulfanilamide 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 4.6 mm in inside 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 1.36 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 4.5 with diluted acetic acid (31) (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 clavularic acid is about 6 minutes.

System suitability—

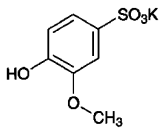
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, clavularic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavularic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Potassium Guaiacolsulfonate

グアヤコールスルホン酸カリウム



$C_7H_7KO_5S$: 242.29

Monopotassium 4-hydroxy-3-methoxybenzenesulfonate [*1321-14-8*]

Potassium Guaiacolsulfonate contains not less than 98.5% of $C_7H_7KO_5S$, calculated on the anhydrous basis.

Description Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in water and in formic acid, soluble in methanol, and practically insoluble in ethanol (95), in acetic anhydride and in diethyl ether.

Identification **(1)** To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution, pH 7.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests ⟨*1.09*⟩ for potassium salt.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

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

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Potassium Guaiacolsulfonate 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak

area obtained from these solutions by the automatic integration method: the total area of peaks other than the peak of potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethylaminopropylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate TS and methanol (20:1).

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

Selection of column: Weigh 50 mg each of potassium guaiacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiacolsulfonate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from 5 μL of the standard solution is not less than 10 mm.

Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

Water ⟨*2.48*⟩ 3.0 – 4.5% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ 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
= 24.23 mg of $C_7H_7KO_5S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Potassium Hydroxide

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of KOH.

Description Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in the presence of moisture.

Identification **(1)** A solution of Potassium Hydroxide (1 in 500) is alkaline.

(2) A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests ⟨*1.09*⟩ for potassium salt.

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

(2) Chloride ⟨*1.03*⟩—Dissolve 2.0 g of Potassium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 8 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 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

(4) Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test ⟨*1.04*⟩ (1): no persistent yellow color develops.

(5) Potassium carbonate—The amount of potassium carbonate (K_2CO_3: 138.21) is not more than 2.0% when calculated by the following equation using *B* (mL) obtained in the Assay.

Amount of potassium carbonate (mg) = 138.21 × *B*

Assay Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate ⟨*2.50*⟩ with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount *A* (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate ⟨*2.50*⟩ again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the amount *B* (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount KOH from the amount, *A* (mL) − *B* (mL).

Each mL of 0.5 mol/L sulfuric acid VS
= 56.11 mg of KOH

Containers and storage Containers—Tight containers.

Potassium Iodide

ヨウ化カリウム

KI: 166.00

Potassium Iodide, when dried, contains not less than 99.0% of KI.

Description Potassium Iodide occurs as colorless or white

crystals, or a white crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It is slightly deliquescent in moist air.

Identification A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests <*1.09*> for potassium salt and for iodide.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color develops.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Iodide 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).

(8) Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test (1) <*1.04*>: a yellow color develops, but does not persist.

(10) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 1.0% (2 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <*2.50*> with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 16.60 mg of KI

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Potassium Permanganate

過マンガン酸カリウム

$KMnO_4$: 158.03

Potassium Permanganate, when dried, contains not less than 99.0% of $KMnO_4$.

Description Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

Identification A solution of Potassium Permanganate (1 in 100) responds to Qualitative Tests <*1.09*> for permanganate.

Purity **(1)** Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color, and dry at 105°C for 2 hours: the mass of the residue is not more than 4 mg.

(2) Arsenic <*1.11*>—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add hydrogen peroxide (30) dropwise until the solution remains colorless, and evaporate on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color.

Standard color: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydrogen peroxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out the test with this solution in the same manner as the test solution (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, silica gel, 18 hours).

Assay Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, dissolve in water to make exactly 200 mL, and use this solution as the sample solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a 500-mL conical flask, add 200 mL of diluted sulfuric acid (1 in 20), and keep at a temperature between 30°C and 35°C. Transfer the sample solution to a buret. Add quickly 23 mL of the sample solution from the buret to the flask while shaking gently, and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55°C and 60°C, and continue the titration <*2.50*> slowly until the red color persists for 30 seconds.

Each mL of 0.05 mol/L oxalic acid VS
= 3.161 mg of $KMnO_4$

Containers and storage Containers—Tight containers.

Potassium Sulfate

硫酸カリウム

K_2SO_4: 174.26

Potassium Sulfate, when dried, contains not less than 99.0% of K_2SO_4.

Description Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste.

It is soluble in water and practically insoluble in ethanol (95).

Identification A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests <*1.09*> for potassium salt and for sulfate.

Purity **(1)** Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water: the solution is clear, colorless and neutral.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium 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).

(4) Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <*1.04*> (1): no persistent yellow color develops.

(5) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of K_2SO_4
= amount (mg) of barium sulfate ($BaSO_4$) × 0.747

Containers and storage Containers—Well-closed containers.

Potato Starch

Amylum Solani

バレイショデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Potato Starch consists of starch granules derived from the tuber of *Solanum tuberosum* Linné (*Solanaceae*).

◆**Description** Potato Starch occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).◆

Identification **(1)** Examined under a microscope <*5.01*> using a mixture of water and glycerin (1:1), Potato Starch presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30 – 100 μm in size but occasionally exceeding 100 μm, or rounded, 10 – 35 μm in size. There are occasional compound granules having two to four components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Potato Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed, and the color disappears by heating.

pH <*2.54*> Put 5.0 g of Potato Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 5.0 and 8.0.

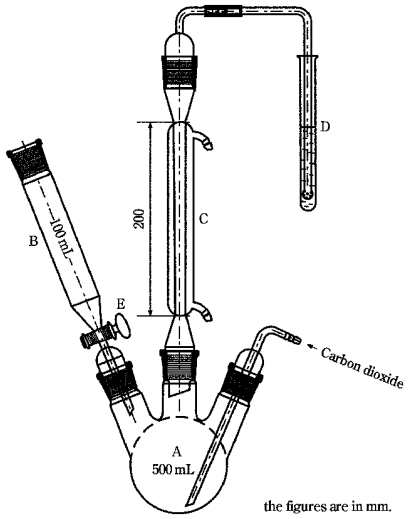
Purity **(1)** Iron—To 1.5 g of Potato Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not darker than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Potato Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <*2.50*> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm,

calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Boiling flask (500 mL)
B: Funnel (100 mL)
C: Condenser
D: Test-tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of the sample
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

◆**(4)** Foreign matter—Under a microscope <5.01>, Potato Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.◆

Loss on drying <2.41> Not more than 20.0% (1 g, 130°C, 90 minutes).

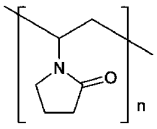
Residue on ignition <2.44> Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers.◆

Povidone

Polyvidone
Polyvinylpyrrolidone

ポビドン



$(C_6H_9NO)_n$
Poly[(2-oxopyrrolidin-1-yl)ethylene]
[*9003-39-8*]

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 25 and not more than 90.

The nominal K-value is shown on the label.

Description Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, in methanol and in ethanol (95), slightly soluble in acetone, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Povidone RS previously dried at 105°C for 6 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Povidone 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) Aldehydes—Weigh accurately about 1.0 g of Povi-

done and dissolve in 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the sample solution. Separately, dissolve 0.100 g of freshly distilled acetaldehyde in water previously cooled to 4°C to make exactly 100 mL. Allow to stand at 4°C for about 20 hours, pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL, and use this solution as the standard solution. Measure 0.5 mL each of the sample solution, standard solution and water (for blank test), transfer to separate cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution, pH 9.0, and 0.2 mL of β-nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 2 to 3 minutes at 22 ± 2°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the control solution. Determine the absorbances, A_{T1}, A_{S1} and A_{B1} of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase solution to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at 22 ± 2°C. Determine the absorbances, A_{T2}, A_{S2} and A_{B2} of these solutions in the same manner as above: the content of aldehydes is not more than 500 ppm (expressed as acetaldehyde).

$$\text{Content (ppm) of aldehydes expressed as acetaldehyde} = \frac{1000}{M} \times \frac{(A_{T2} - A_{T1}) - (A_{B2} - A_{B1})}{(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})}$$

M: Amount (g) of Povidone, calculated on the anhydrous basis

(4) 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in diluted methanol (1 in 5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of 1-vinyl-2-pyrrolidone in each solution: the content of 1-vinyl-2-pyrrolidone is not more than 10 ppm.

$$\text{Content (ppm) of 1-vinyl-2-pyrrolidone} = 2.5/M \times A_T/A_S$$

M: Amount (g) of Povidone, calculated on the anhydrous basis

Operating conditions—

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

Column: Stainless steel columns about 4 mm in inside diameter and about 25 mm in length, and about 4 mm in inside diameter and about 250 mm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter), and use them as a guard column and a separation column, respectively.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 10 minutes.

Selection of column: Dissolve 0.01 g of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add diluted methanol (1 in 5) to make 100 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 1-vinyl-2-pyrrolidone and vinyl acetate in this order with the resolution between these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 50 μL of the standard solution is between 10 mm and 15 mm.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of obtained peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Washing of the guard column: After each test with the sample solution, wash away the polymeric material of Povidone from the guard column by passing the mobile phase through the column backwards for about 30 minutes at the same flow rate as applied in the test.

(5) Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared by adding 2 mL of 13% sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the subsequent solution of the sample solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

(6) Hydrazine—Transfer 2.5 g of Povidone to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 μL of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 0.09 g of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate coated with a 0.25-mm layer of dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the *R*f value of the fluorescent spot from the standard solution is about 0.3, and the fluorescence of the spot from the sample solution corresponding to the spot from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

Water <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

K-value Weigh accurately an amount of Povidone, equivalent to 1.00 g calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method 1 under Viscosity Determination <*2.53*>, and calculate the K-value by the following formula.

$$K = \frac{1.5 \log \eta_{rel} - 1}{0.15 + 0.003\,c} + \frac{\sqrt{300\,c \log \eta_{rel} + (c + 1.5\,c \log \eta_{rel})^2}}{0.15\,c + 0.003\,c^2}$$

c: Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis

η_{rel}: Kinematic viscosity of the sample solution relative to that of water

The K-value of Povidone is not less than 90% and not more than 108% of the nominal K-value.

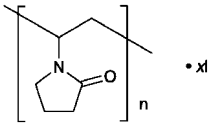
Assay Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium slfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a carbonaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (2 in 5) through the funnel, rinse cautiously the funnel with 10 ml of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to get 80 to 100 mL of the distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate <*2.50*> the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS
= 0.700 mg of N

Containers and storage Containers—Tight containers.

Povidone-Iodine

ポビドンヨード



$(C_6H_9NO)_n.xI$

Poly[(2-oxopyrrolidin-1-yl)ethylene] iodine

[*25655-41-8*]

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than 9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

Description Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

Identification **(1)** To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

(2) To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiosulfate TS, and add 1 mL of ammonium thiocyanate-cobalt (II) nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

Purity **(1)** Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Povidone-Iodine 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 <*1.11*>—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

(4) Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrogensulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: it is not more than 6.6%.

Loss on drying <*2.41*> Not more than 8.0% (1 g, 100°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (5 g).

Assay **(1)** Available iodine—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 30 mL of water, and titrate ⟨*2.50*⟩ with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

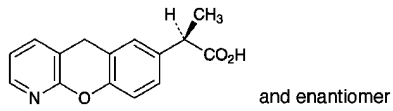
Each mL of 0.02 mol/L sodium thiosulfate VS
= 2.538 mg of I

(2) Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩.

Containers and storage Containers—Tight containers.

Pranoprofen

プラノプロフェン



and enantiomer

$C_{15}H_{13}NO_3$: 255.27
(2*RS*)-2-(10*H*-9-Oxa-1-azaanthracen-6-yl)propanoic acid
[*52549-17-4*]

Pranoprofen, when dried, contains not less than 98.5% of $C_{15}H_{13}NO_3$.

Description Pranoprofen occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranoprofen in *N*,*N*-dimethylformamide (1 in 30) shows no optical rotation.

Identification **(1)** Dissolve 0.02 g of Pranoprofen in 1 mol/L hydrochloric acid TS to make 100 mL, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pranoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 186 – 190°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Pranoprofen in 40 mL of methanol, and 6 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 as follows. To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pranoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of the Standard Lead Solution (not more than 10 ppm).

(3) Related Substances—Dissolve 50 mg of Pranoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than the peak of pranoprofen from the sample solution is not larger than the peak area of pranoprofen from the standard solution, and the total peak area of them is not larger than 2 times the peak area of pranoprofen from the standard solution.

Operating conditions—

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

Column: A stainless steel column about 6 mm in inside 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 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

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

Selection of column: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranoprofen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranoprofen.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

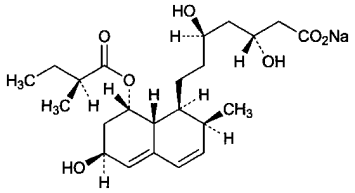
Assay Weigh accurately about 0.4 g of Pranoprofen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.53 mg of $C_{15}H_{13}NO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pravastatin Sodium

プラバスタチンナトリウム



$C_{23}H_{35}NaO_7$: 446.51
Monosodium (3*R*,5*R*)-3,5-dihydroxy-7-{(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[(2*S*)-2-methylbutanoyloxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}heptanoate
[*81131-70-6*]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of $C_{23}H_{35}NaO_7$, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Pravastatin Sodium occurs as a white to yellowish white, powder or crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Pravastatin Sodium (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2970 cm^{-1}, 2880 cm^{-1}, 1727 cm^{-1} and 1578 cm^{-1}.

(3) Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium 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 <*2.03*>. Spot 2 μL each of the sample 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, ethanol (99.5) and acetic acid (100) (80:16:1) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the color tone and the *R*f value of the principal spot with the sample solution are not different with them of the spot with the standard solution.

(4) A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*>: +153 – +159° (0.1 g calculated on the anhydrous basis and corrected on the amount of residual solvent, water, 20 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pravastatin Sodium 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 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and standard solution at not over than15°C.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 5 mg of pravastatin sodium in 50 mL of the mixture of water and methanol (11:9). When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

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

(3) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (previously determine the water <*2.48*> with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained as the standard solution. Per-

form the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{35}NaO_7 = M_S \times Q_T/Q_S \times 4 \times 1.052$$

M_S: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, methanol, acetic acid (100) and triethylamine (550:450:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 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 pravastatin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pravastatin Sodium Fine Granules

プラバスタチンナトリウム細粒

Pravastatin Sodium Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation Prepare fine particles as directed under Granules, with Pravastatin Sodium.

Identification To an amount of Pravastatin Sodium Fine Granules, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 237 nm and 241 nm.

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 5°C after preparation. To an amount of Pravastatin Sodium Fine Granules, equivalent to 25 mg of Pravastatin Sodium according to the labeled amount, add 25 mL of a mixture of water and methanol (1:1), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time of about 0.36 and about 1.9 to pravastatin, obtained from the sample solution is not larger than 1/2 times and 3 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 4.5 times the peak area of pravastatin from the standard solution. For this calculation, use the area of peaks, obtained by automatic integration method of related substances having the relative retention time of about 0.36, about 0.28 and about 0.88 to pravastatin, after multiplying by their relative response factors, 0.58, 0.86 and 0.82, respectively.

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside 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: A mixture of water, methanol, acetic acid (100) and triethylamine (750:250:1:1).

Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin 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 con-

ditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: the Pravastatin Sodium Fine Granules in single-unit container meets the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Pravastatin Sodium Fine Granules add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate add a mixture of water and methanol (1 in 1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$= M_S \times Q_T/Q_S \times V/100 \times 1.052$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Pravastatin Sodium Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 238 nm and A_{T2} and A_{S2} at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$= M_S/M_T \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2})$
$\times 1/C \times 27 \times 0.806$

M_S: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

M_T: Amount (g) of sample

C: Labeled amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$) in 1 g

Particle size ⟨*6.03*⟩ It meets the requirements of Fine granules.

Assay Weigh accurately an amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 20 mL of the internal standard solution, agitate for 15 minute with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$= M_S \times Q_T/Q_S \times 1/5 \times 1.052$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

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 pravastatin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Container—Well-closed containers.

Pravastatin Sodium Solution

プラバスタチンナトリウム液

Pravastatin Sodium Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation Prepare as directed under Liquids

and Solutions for Oral Administration, with Pravastatin Sodium.

Identification Pass a volume of Pravastatin Sodium Solution, equivalent to 1 mg of Pravastatin Sodium according to the labeled amount, through a column [5.5 mm in inside diameter, packed with 30 mg of divinylbenzene-*N*-vinyl pyrrolidone copolymer for column chromatography (30 μm in particle size), and washed with 1 mL of methanol and 1 mL of water]. Then wash with 1 mL of water, and elute with 1 mL of methanol. To 0.1 mL of the eluate add water to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 237 nm and 241 nm.

pH Being specified separately.

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of Pravastatin Sodium according to the labeled amount, add a mixture of methanol and water (5:3) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time about 0.24 and about 0.85 to pravastatin, obtained from the sample solution is not larger than 2 times the peak area of pravastatin from the standard solution, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 3/10 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 3.5 times the peak area of pravastatin 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.

Time span of measurement: About 2 times as long as the retention time of pravasatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 10 μL of this solution is equivalent to 15 to 25% 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 pravastatin are not less than 3400 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.5%.

Uniformity of dosage units <*6.02*> The solution in single-unit container meets the requirement of the Mass variation test.

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^1 CFU/mL, respectively. *Escherichia coli* is not observed.

Assay To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <*2.48*> in the same manner as Pravastatin Sodium), and dissolve in a solution of disodium hydrogen phosphate dodecahydrate (1 in 200) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

$$\text{Amount (mg) of pravastatin sodium} = M_S \times Q_T/Q_S \times 3/25 \times 1.052$$

M_S: Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (3 in 10,000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 3.9 mm in inside 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 30°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (500:500:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 20 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 pravastatin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Container—Tight containers.

Pravastatin Sodium Tablets

プラバスタチンナトリウム錠

Pravastatin Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation Prepare as directed under Tablets, with Pravastatin Sodium.

Identification To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 237 nm and 241 nm.

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 50 mg of Pravastatin Sodium according to the labeled amount, add 40 mL of a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, then add a mixture of water and methanol (1:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time about 0.36 and about 1.9 to pravastatin obtained from the sample solution is not larger than 3/10 times and 2 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 3 times the peak area of pravastatin from the standard solution. For this calculation, use the area of the peaks, having the relative retention time about 0.36, about 0.28 and about 0.88 to pravastatin, after multiplying by their relative response factors, 0.58, 0.86 and 0.82, respectively.

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside 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: A mixture of water, methanol, acetic acid (100) and triethylamine (750:250:1:1).

Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin 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 pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pravastatin Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of pravastatin sodium } (C_{23}H_{35}NaO_7) = M_S \times Q_T/Q_S \times V/100 \times 1.052$$

M_S: Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Pravastatin Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Pravastatin Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.5 μg of pravastatin ($C_{23}H_{36}O_7$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin

1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48> in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 238 nm and A_{T2} and A_{S2} at 256 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 1/C \times 27 \times 0.806$

M_S: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

C: Labeled amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Pravastatin Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 40 mL of the internal standard solution, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48> in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$= M_S \times Q_T/Q_S \times 2/5 \times 1.052$

M_S: Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

System suitability—

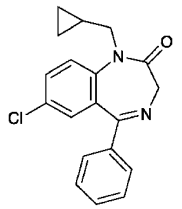
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Container—Well-closed containers.

Prazepam

プラゼパム



$C_{19}H_{17}ClN_2O$: 324.80
7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*2955-38-6*]

Prazepam, when dried, contains not less than 98.5% of $C_{19}H_{17}ClN_2O$.

Description Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 0.01 g of Prazepam in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Prazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the Flame Coloration Tests <1.04> (2) with Prazepam: a green color appears.

Melting point <2.60> 145 – 148°C

Purity **(1)** Chloride <1.03>—To 1.0 g of Prazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. 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) Sulfate <1.14>—To 20 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 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Praze-

pam 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Prazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Prazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample 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 and acetone (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Prazepam, previously dried, dissolve in 60 mL of acetic anhydride, and titrate ⟨*2.50*⟩ 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
= 32.48 mg of $C_{19}H_{17}ClN_2O$

Containers and storage Containers—Tight containers.

Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$: 324.80).

Method of preparation Prepare as directed under Tablets, with Prazepam.

Identification **(1)** To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam according to the labeled amount, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2) To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution medium, the dissolution rate in 30 minutes of Prazepam Tablets is not less than 80%.

Start the test with 1 tablet of Prazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, measure exactly the subsequent V mL of the filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5 μg of prazepam ($C_{19}H_{17}ClN_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C for 2 hours, add 200 mL of the dissolution medium and dissolve with shaking, or by ultrasonication if necessary, add the dissolution medium to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of prazepam for assay
C: Labeled amount (mg) of prazepam ($C_{19}H_{17}ClN_2O$) in 1 tablet

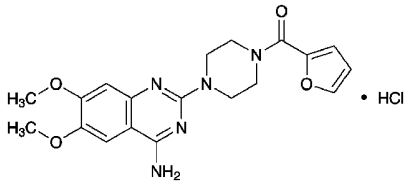
Assay Weigh accurately not less than 20 Prazepam Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam ($C_{19}H_{17}ClN_2O$), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants liquid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 6.496 mg of $C_{19}H_{17}ClN_2O$

Containers and storage Containers—Tight containers.

Prazosin Hydrochloride

プラゾシン塩酸塩



$C_{19}H_{21}N_5O_4$.HCl: 419.86
1-(4-Amino-6,7-dimethoxy-quinazolin-2-yl)-4-(2-furoyl)piperazine monohydrochloride
[*19237-84-4*]

Prazosin Hydrochloride, when dried, contains not less than 97.0% and not more than 103.0% of $C_{19}H_{21}N_5O_4$.HCl.

Description Prazosin Hydrochloride occurs as a white crystalline powder.

It is slightly soluble in methanol, very slightly soluble in ethanol (99.5) and practically insoluble in water.

It gradually turns pale yellowish white on exposure to light.

Melting point: about 270°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Prazosin Hydrochloride in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Prazosin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Prazosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Prazosin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Prazosin Hydrochloride add 5 mL of water and 1 mL of ammonia TS, shake, allow to stand for 5 minutes, and filter. Render the filtrate acid with acetic acid (100): the solution responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Prazosin Hydrochloride 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 Prazosin Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prazosin from the sample solution is not larger than 2 times the peak area of prazosin from the standard solution, and the total area of the peaks other than the peak of prazosin from the sample solution is not larger than 5 times the peak area of prazosin from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 3.484 g of sodium 1-pentane sulfonate and 18 mL of tetramethylammonium hydroxide in 900 mL of water, adjust the pH to 5.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prazosin is about 9 minutes.

Time span of measurement: About 6 times as long as the retention time of prazosin.

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 prazosin obtained from 20 μL of this solution is equivalent to 35 to 65% of that of prazosin 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 prazosin are not less than 4000 and not more than 2.0, respectively.

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

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 25 mg each of Prazosin Hydrochloride and Prazosin Hydrochloride RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, and add a mixture of methanol and water (7:3) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of prazosin in each solution.

Amount (mg) of prazosin hydrochloride ($C_{19}H_{21}N_5O_4$.HCl) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Prazosin Hydrochloride RS

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diame-

ter and 25 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 methanol, water, acetic acid (100) and diethylamine (3500:1500:50:1).

Flow rate: Adjust the flow rate so that the retention time of prazosin 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 and the symmetry factor of the peak of prazosin are not less than 5000 and not more than 2.0, respectively.

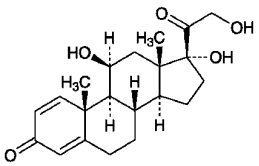
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prednisolone

プレドニゾロン



$C_{21}H_{28}O_5$: 360.44

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione

[*50-24-8*]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of $C_{21}H_{28}O_5$.

Description Prednisolone occurs as a white, crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate and in chloroform, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

Identification (1) To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Prednisolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Prednisolone and Prednisolone RS in ethyl acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +113 – +119° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity (1) Selenium—To 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light 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 sample 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 sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine constant absorbances, 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 or argon.

(2) Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample solution.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Dissolve about 25 mg each of Prednisolone and Prednisolone RS, previously dried, and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone to that of the internal standard.

$$\text{Amount (mg) of } C_{21}H_{28}O_5 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Prednisolne RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 247 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with fluorosilanized 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 and methanol (13:7).

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

System suitability—

System performance: Dissolve 25 mg of Prednisolone and 25 mg of hydrocortisone in 100 mL of methanol. 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, hydrocortisone and prednisolone 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Tablets

プレドニゾロン錠

Prednisolone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

Method of preparation Prepare as directed under Tablets, with Prednisolone.

Identification **(1)** Weigh a quantity of powdered Prednisolone Tablets, equivalent to 0.05 g of Prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, and add methanol to make exactly V' mL to provide a solution that contains about 10 μg of prednisolone ($C_{21}H_{28}O_5$) per ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add 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 standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{18}O_5) = M_S \times A_T/A_S \times V'/V \times 1/10$$

M_S: Amount (mg) of Prednisolone RS

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Prednisolone Tablets is not less than 70%.

Start the test with 1 tablet of Prednisolone Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) 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, of the sample solution and standard solution at the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of prednisolone } (C_{21}H_{28}O_5) = M_S \times A_T/A_S \times 1/C \times 45$$

M_S: Amount (mg) of Prednisolone RS

C: Labeled amount (mg) of prednisolone ($C_{21}H_{28}O_5$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone ($C_{21}H_{28}O_5$), add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.

Amount (mg) of prednisolone ($C_{21}H_{28}O_5$)
$= M_S \times Q_T/Q_S \times 1/5$

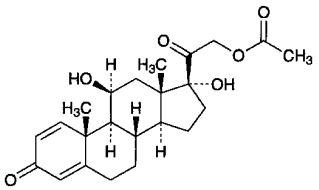
M_S: Amount (mg) of Prednisolone RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Containers and storage Containers—Tight containers.

Prednisolone Acetate

プレドニゾロン酢酸エステル



$C_{23}H_{30}O_6$: 402.48
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-acetate
[*52-21-1*]

Prednisolone Acetate, when dried, contains not less than 96.0% and not more than 102.0% of $C_{23}H_{30}O_6$.

Description Prednisolone Acetate occurs as a white, crystalline powder.

It is slightly soluble in methanol, in ethanol (95), in ethanol (99.5), and in chloroform, and practically insoluble in water.

Melting point: about 235°C (with decomposition).

Identification **(1)** To 2 mg of Prednisolone Acetate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectra of Prednisolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum in a range between 4000 cm^{-1} and 650 cm^{-1} with the Infrared Reference Spectrum or the spectrum of previously dried Prednisolone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethanol (99.5), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +128 – +137° (after drying, 70 mg, methanol, 20 mL, 100 mm).

Purity Related substanes—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1). Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (wavelength: 254 mm): the spots from the sample solution corresponding to those from the standard solution are not more intense than the spots from the standard solution, and any spot from the sample solution other than the principal spot and the spots from prednisolone, cortisone acetate and hydrocortisone acetate does not appear.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate RS, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the text with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of prednisolone acetate to that of the internal standard.

Amount (mg) of $C_{23}H_{30}O_6 = M_S \times Q_T/Q_S$

M_S: Amount (mg) of Prednisolone Acetate RS

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside 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 water and acetonitrile (3:2).

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

System suitability—

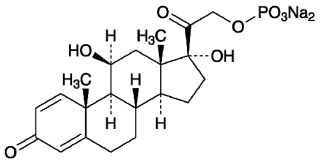
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Sodium Phosphate

プレドニゾロンリン酸エステルナトリウム



$C_{21}H_{27}Na_2O_8P$: 484.39
11β,17,21-Trihydroxypregna-1,4-diene 3,20-dione
21-(disodium phosphate)
[*125-02-0*]

Prednisolone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of $C_{21}H_{27}Na_2O_8P$, calculated on the anhydrous basis.

Description Prednisolone Sodium Phosphate occurs as a white to pale yellow powder.

It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Moisten 1.0 g of Prednisolone Sodium Phosphate with a small amount of sulfuric acid, and gradually heat to incinerate. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests <*1.09*> for phosphate.

(2) Dissolve 2 mg of Prednisolone Sodium Phosphate in 2 mL of sulfuric acid, and allow to stand for 2 minutes: a deep red color, without fluorescence, develops.

(3) Determine the absorption spectrum of a solution of Prednisolone Sodium Phosphate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Prednisolone Sodium Phosphate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) The solution obtained in (1) responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +96 – +103° (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Prednisolone Sodium Phosphate in 100 mL of water: the pH of the solution is between 7.5 and 9.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Prednisolone Sodium Phosphate in 10 mL of water: the solution is clear and not more colored than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make 10 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 0.5 g of Prednisolone Sodium Phosphate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.25 g of Prednisolone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Phosphoric Acid Standard Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphtol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of each solution from the sample solution and standard solution at 740 nm: the content of free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of Prednisolone Sodium Phosphate, calculated on the anhydrous basis

(4) Related substances—Dissolve 10 mg of Prednisolone Sodium Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prednisolone phosphate from the sample solution is not larger than 1.5 times the peak area of prednisolone phosphate from the standard solution, and the total area of the peaks other than the peak of prednisolone phosphate from the sample solution is not larger than 2.5 times the peak area of prednisolone phosphate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 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 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid. To 1000 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of prednisolone phosphate is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of prednisolone phosphate.

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 prednisolone phosphate ob-

tained from 20 μL of this solution is equivalent to 7 to 13% of that of prednisolone phosphate 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 prednisolone phosphate are not less than 3000 and not more than 2.0, respectively.

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

(5) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Prednisolone Sodium Phosphate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add 1 mL of alkaline phosphatase TS, and allow to stand for 2 hours with occasional shaking. To this solution add exactly 20 mL of 1-octanol, and shake vigorously. Centrifuge this solution, pipet 10 mL of the 1-octanol layer, add 1-octanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in 1-octanol to make exactly 100 mL. Pipet 6 mL of this solution, add a solution prepared by adding 1 mL of alkaline phosphatase TS to 2 mL water and being allowed to stand for 2 hours with occasional gentle shaking, add exactly 14 mL of 1-octanol, and shake vigorously. Proceed in the same manner as the sample solution to make the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using 1-octanol as the blank, and determine the absorbances, A_T and A_S, at 245 nm.

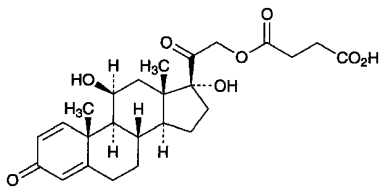
Amount (mg) of prednisolone sodium phosphate ($C_{21}H_{27}Na_2O_8P$)
$= M_S \times A_T/A_S \times 3 \times 1.344$

M_S: Amount (mg) of Prednisolone RS

Containers and storage Containers—Tight containers.

Prednisolone Succinate

プレドニゾロンコハク酸エステル



$C_{25}H_{32}O_8$: 460.52
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(hydrogen succinate)
[*2920-86-7*]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{32}O_8$.

Description Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

Melting point: about 205°C (with decomposition).

Identification **(1)** To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +114 – +120° (after drying, 67 mg, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the 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 ⟨*2.03*⟩. Spot 5 μL of the sample 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 and ethanol (95) (2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg each of Prednisolone Succinate and Prednisolone Succinate RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

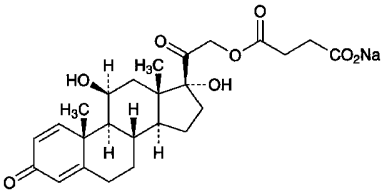
Amount (mg) of $C_{25}H_{32}O_8 = M_S \times A_T/A_S$

M_S: Amount (mg) of Prednisolone Succinate RS

Containers and storage Containers—Tight containers.

Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム



$C_{25}H_{31}NaO_8$: 482.50
Monosodium 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate
[*1715-33-9*]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before used.

It contains not less than 72.4% and not more than 83.2% of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$), and the equivalent of not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

The amount should be stated as the amount of prednisolone ($C_{21}H_{28}O_5$).

Method of preparation Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

Description Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

Identification **(1)** To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisolone Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on drying <*2.41*> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 2.4 EU/mg of prednisolone ($C_{21}H_{28}O_5$).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone ($C_{21}H_{28}O_5$), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate RS, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography according <*2.01*> to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone succinate to that of the internal standard.

Amount (mg) of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$)
$= M_S \times Q_T/Q_S \times 5 \times 1.048$

Amount (mg) of prednisolone ($C_{21}H_{28}O_5$)
$= M_S \times Q_T/Q_S \times 5 \times 0.783$

M_S: Amount (mg) of Prednisolone Succinate RS

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of disodium hydrogen phosphate dodacahydrate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.

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

System suitability—

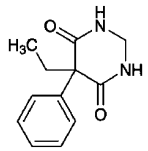
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Primidone

プリミドン



$C_{12}H_{14}N_2O_2$: 218.25

5-Ethyl-5-phenyl-2,3-dihyropyrimidine-4,6(1*H*,5*H*)-dione

[*125-33-7*]

Primidone, when dried, contains not less than 98.5% of $C_{12}H_{14}N_2O_2$.

Description Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste.

It is soluble in *N*,*N*-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

(2) Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

Melting point ⟨*2.60*⟩ 279 – 284°C

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Primidone in 10 mL of *N*,*N*-dimethylformamide: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Primidone 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) 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μL of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: Q_T is not more than Q_S.

Internal standard solution—A solution of stearylalcohol in pyridine (1 in 2000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen.

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

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating condition, 2-ethyl-2-phenylmalonediamide 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 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg each of Primidone and Primidone RS, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbance, A_1, of the sample solution and standard solution at the wavelength of maximum absorption at about 257 nm, and the absorbances, A_2 and A_3, at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using ethanol (95) as the blank.

$$\text{Amount (mg) of } C_{12}H_{14}N_2O_2 = M_S \times (2A_1 - A_2 - A_3)_T/(2A_1 - A_2 - A_3)_S$$

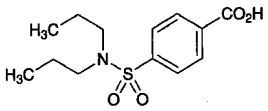
M_S: Amount (mg) of Primidone RS

where, $(2A_1 - A_2 - A_3)_T$ is the value from the sample solution, and $(2A_1 - A_2 - A_3)_S$ is from the standard solution.

Containers and storage Containers—Tight containers.

Probenecid

プロベネシド



$C_{13}H_{19}NO_4S$: 285.36
4-(Dipropylaminosulfonyl)benzoic acid
[*57-66-9*]

Probenecid, when dried, contains not less than 98.0% of $C_{13}H_{19}NO_4S$.

Description Probenecid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

It is sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: 198 – 200°C

Identification **(1)** Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectrum of a solution of Probenecid in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Acidity—To 2.0 g of Probenecid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Chloride <*1.03*>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of nitric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate 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) Sulfate <*1.14*>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of hydrochloric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate 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 <*1.07*>—Proceed with 2.0 g of Probenecid 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 <*1.11*>—Prepare the test solution with 1.0 g of Probenecid according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Probenecid, previously dried, and dissolve in 50 mL of neutralized ethanol. Titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 28.54 mg of $C_{13}H_{19}NO_4S$

Containers and storage Containers—Well-closed containers.

Probenecid Tablets

プロベネシド錠

Probenecid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$: 285.36).

Method of preparation Prepare as directed under Tablets, with Probenecid.

Identification **(1)** Weigh a quantity of powdered Probenecid Tablets, equivalent to 0.5 g of Probenecid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts <*2.60*> between 196°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Probenecid Tablets add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, treat with ultrasonic waves with occasional shaking to disintegrate the tablet completely, and add ethanol (99.5) to make exactly 100 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly V mL so that each mL contains about 15 μg of probenecid ($C_{13}H_{19}NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid RS, previously dried at 105°C for 4 hours, dissolve in 15 mL of water, 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared by adding

ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, as the blank, and determine the absorbances, A_T and A_S, at 248 nm.

Amount (mg) of probenecid ($C_{13}H_{19}NO_4S$)
$= M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of Probenecid RS

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Probenecid Tablets is not less than 80%.

Start the test with 1 tablet of Probenecid Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 14 μg of probenecid ($C_{13}H_{19}NO_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probenecid RS, previously dried at 105°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 244 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of probenecid ($C_{13}H_{19}NO_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Probenecid RS
C: Labeled amount (mg) of probenecid ($C_{13}H_{19}NO_4S$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Probenecid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probenecid ($C_{13}H_{19}NO_4S$), add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, shake, add 30 mL of ethanol (99.5), disperse the particles with the aid of ultrasonic waves, and add ethanol (99.5) to make exactly 100 mL. Centrifuge the solution, pipet 3 mL of the supernatant liquid, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid RS, previously dried at 105°C for 4 hours, add 15 mL of water and 1 mL of 1 mol/L hydrochloric acid TS, then add ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared by mixing 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient ethanol (99.5) to make exactly 50 mL, as the blank.

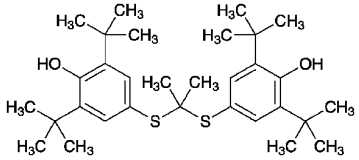
Amount (mg) of probenecid ($C_{13}H_{19}NO_4S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Probenecid RS

Containers and storage Containers—Well-closed containers.

Probucol

プロブコール



$C_{31}H_{48}O_2S_2$: 516.84
4,4′-[Propan-2,2-diylbis(sulfandiyl)]bis[2,6-bis(1,1-dimethylethyl)phenol]
[*23288-49-5*]

Probucol, when dried, contains not less than 98.5% and not more than 101.0% of $C_{31}H_{48}O_2S_2$.

Description Probucol occurs as a white crystalline powder.

It is very soluble in tetrahydrofuran, freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water.

It gradually turns light yellow on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Probucol in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probucol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Probucol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Probucol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 125 – 128°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Probucol 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. Dissolve 0.40 g of Probucol in 5 mL of ethanol (99.5), add the mobile phase to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 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 exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.9 with respect to probucol from the sample solution is not larger than the peak area of probucol from the standard solution; the area of peak hav-

ing the relative retention time of about 1.9 with respect to probucol from the sample solution is not larger than 25 times the peak area of probucol from the standard solution; and the area of each peak other than the peak of probucol and other than the peaks mentioned above is not larger than 5 times the peak area of probucol from the standard solution. Furthermore, the total area of the peaks other than probucol from the sample solution is not larger than 50 times the peak area of probucol from the standard solution. For this calculation, use the areas of the peaks, having the relative retention times of about 0.9 and about 1.9 with respect to probucol, after multiplying by their relative response factors, 1.2 and 1.4, respectively.

Operating conditions—

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

Time span of measurement: About 3 times as long as the retention time of probucol, beginning after the solvent peak, excluding the peak having the relative retention time of about 0.5 with respect to probucol.

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 probucol obtained from 5 μL of this solution is equivalent to 14 to 26% of that of probucol from 5 μL of the standard solution.

System performance: To 1 mL of the sample solution add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of a solution of phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) in the mobile phase (1 in 1000), 5 mL of ethanol (99.5), and the mobile phase to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) and probucol 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of probucol is not more than 5%.

(3) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 80°C, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg each of Probucol and Probucol RS, previously dried, dissolve each in 5 mL of tetrahydrofuran, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Probucol RS

Internal standard solution—Dissolve 0.2 g of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in 1 mL of tetrahydrofuran, and add the mobile phase to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: A mixture of acetonitrile and water (93:7).

Flow rate: Adjust the flow rate so that the retention time of probucol is about 13 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 probucol 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Probucol Fine Granules

プロブコール細粒

Probucol Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of probucol ($C_{31}H_{48}O_2S_2$: 516.84).

Method of preparation Prepare as directed under Granules, with Probucol.

Identification To an amount of pulverized Probucol Fine Granules, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 240 nm and 244 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: the granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Probucol Fine Granules add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet V mL of the supernatant liquid, equivalent to about 5 mg of probucol ($C_{31}H_{48}O_2S_2$), add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = M_S \times Q_T/Q_S \times 10/V$$

M_S: Amount (mg) of Probucol RS

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Particle size ⟨*6.03*⟩ It meets the requirements of Fine granules.

Assay Weigh accurately an amount of pulverized Probucol Fine Granules, equivalent to about 0.25 g of probucol ($C_{31}H_{48}O_2S_2$), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of Probucol RS

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Probucol.

Column: A stainless steel column 4.6 mm in inside diameter and 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 10 μL of the standard solution under the above operating conditions, the internal standard and probucol 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Probucol Tablets

プロブコール錠

Probucol Tablets contain not less than 95.0% and not more than 105.0% of probucol ($C_{31}H_{48}O_2S_2$: 516.84).

Method of preparation Prepare as directed under Tablets, with Probucol.

Identification To an amount of pulverized Probucol Tablets, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 240 nm and 244 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Shake 1 tablet of Probucol Tablets with a suitable amount of methanol until the tablet is disintegrated, and add methanol to make exactly V mL so that each mL of the solution contains about 2.5 mg of probucol ($C_{31}H_{48}O_2S_2$). Centrifuge the solution, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = M_S \times Q_T/Q_S \times V/20$$

M_S: Amount (mg) of Probucol RS

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Disintegration ⟨*6.09*⟩ It meets the requirement.

Assay Weigh accurately the mass of 20 Probucol Tablets, and powder the tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probucol ($C_{31}H_{48}O_2S_2$), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of Probucol RS

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Probucol.

Column: A stainless steel column 4.6 mm in inside diameter and 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 10 μL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 3.

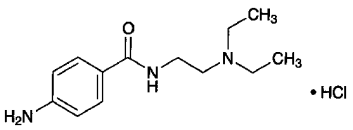
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Procainamide Hydrochloride

プロカインアミド塩酸塩



$C_{13}H_{21}N_3O.HCl$: 271.79
4-Amino-*N*-(2-diethylaminoethyl)benzamide monohydrochloride
[*614-39-1*]

Procainamide Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of $C_{13}H_{21}N_3O.HCl$.

Description Procainamide Hydrochloride occurs as a white to light yellow crystalline powder.

It is very soluble in water and soluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Procainamide Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

Melting point <*2.60*> 165 – 169°C

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

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Procainamide 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 <*1.11*>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 procainamide from the sample solution is not larger than the peak area of procainamide from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and methanol (9:1).

Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of procainamide.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide obtained from 10 μL of this solution is equivalent to 40 to 60% of that of procainamide 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 the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.

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

Loss on drying <*2.41*> Not more than 0.3% (2 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

Containers and storage Containers—Tight containers.

Procainamide Hydrochloride Injection

プロカインアミド塩酸塩注射液

Procainamide Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$: 271.79).

Method of preparation Prepare as directed under Injections, with Procainamide Hydrochloride.

Description Procainamide Hydrochloride Injection is a clear, colorless or light yellow liquid.

pH: 4.0 – 6.0

Identification **(1)** To a volume of Procainamide Hydrochloride Injection, equivalent to 10 mg of Procainamide Hydrochloride according to the labeled amount, add 1 mL of dilute hydrochloric acid and water to make 5 mL: the solution responds to the Qualitative Tests <*1.09*> (1) for primary aromatic amines.

(2) To a volume of Procainamide Hydrochloride Injection, equivalent to 0.1 g of Procainamide Hydrochloride according to the labeled amount, add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 281 nm.

(3) Procainamide Hydrochloride Injection responds to the Qualitative Tests <*1.09*> (2) for chloride.

Bacterial endotoxins <*4.01*> Less than 0.30 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dilute an accurately measured volume of Procainamide Hydrochloride Injection, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl), with 5 mL of hydrochloric acid and water to 50 mL, add 10 mL of potassium bromide solution (3 → 10), cool to 15°C or lower, and titrate <*2.50*> with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O$.HCl

Containers and storage Containers—Hermetic containers.

Procainamide Hydrochloride Tablets

プロカインアミド塩酸塩錠

Procainamide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl: 271.79).

Method of preparation Prepare as directed under Tablets, with Procainamide Hydrochloride.

Identification To a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride according to the labeled amount, add 30 mL of water, shake well, filter, and use the filtrate as the sample solution. To 0.2 mL of the sample solution add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Procainamide Hydrochloride Tablets add $3V/5$ mL of 0.02 mol/L phosphate buffer solution, pH 3.0, treat with ultrasonic waves to disintegrate the tablet completely, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly V mL so that each mL contains about 2.5 mg of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl), and shake for 5 minutes. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 250 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl)
$= M_S \times A_T/A_S \times V/20$

M_S: Amount (mg) of procainamide hydrochloride for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Procainamide Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Procainamide Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 7 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 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. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 278 nm.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of procainamide hydrochloride for assay

C: Labeled amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl) in 1 tablet

Assay To 10 Procainamide Hydrochloride Tablets add about 300 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, and treat with ultrasonic waves to disintegrate the tablets completely. To this solution add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 500 mL, and stir for 5 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, and add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly V' mL so that each mL contains about 10 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl). Filter this solution 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 sample solution. Separately, weigh accurately about 50 mg of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of procainamide in each solution.

Amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/10$

M_S: Amount (mg) of procainamide hydrochloride for assay

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and methanol (9:1).

Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

System suitability—

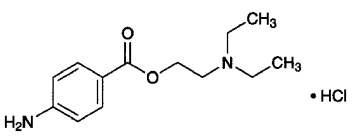
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.

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

Containers and storage Containers—Tight containers.

Procaine Hydrochloride

プロカイン塩酸塩



$C_{13}H_{20}N_2O_2.HCl$: 272.77
2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride [*51-05-8*]

Procaine Hydrochloride, when dried, contains not less than 99.0% of $C_{13}H_{20}N_2O_2.HCl$.

Description Procaine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Procaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procaine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution prepared by dissoluing 1.0 g of Procaine Hydrochloride in 20 mL of water is between 5.0 and 6.0.

Melting point <*2.60*> 155 – 158°C

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Procaine 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—To 1.0 g of Procaine Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dibutyl ether, *n*-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate more at 105°C for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. The principal spot from the sample solution stays at the origin.

Loss on drying <*2.41*> Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate <*2.50*> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
$= 27.28$ mg of $C_{13}H_{20}N_2O_2.HCl$

Containers and storage Containers—Well-closed contain-

ers.

Procaine Hydrochloride Injection

プロカイン塩酸塩注射液

Procaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl: 272.77).

Method of preparation Prepare as directed under Injections, with Procaine Hydrochloride.

Description Procaine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To a volume of Procaine Hydrochloride Injection, equivalent to 0.01 g of Procaine Hydrochloride according to the labeled amount, add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 219 nm and 223 nm, and between 289 nm and 293 nm.

(2) Procaine Hydrochloride Injection responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

pH ⟨*2.54*⟩ 3.3 – 6.0

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.02 EU/unit. Apply to the preparations intended for intraspinal administration.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of procaine hydrochloride to that of the internal standard.

Amount (mg) of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl)
$= M_S \times Q_T/Q_S \times 2/5$

M_S: Amount (mg) of procaine hydrochloride for assay

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

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

Column: A stainless steel column about 6 mm in inside 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: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

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

System suitability—

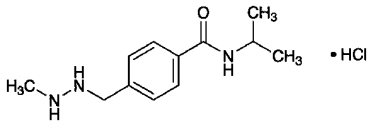
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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

Containers and storage Containers—Hermetic containers.

Procarbazine Hydrochloride

プロカルバジン塩酸塩



$C_{12}H_{19}N_3O$.HCl: 257.76
N-(1-Methylethyl)-
4-[(2-methylhydrazino)methyl]benzamide
monohydrochloride
[*366-70-1*]

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{12}H_{19}N_3O$.HCl.

Description Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

Identification (1) Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.

(2) Determine the absorption spectrum of a solution of Procarbazine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Procarbazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Procarbazine Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 0.10 g of Procarbazine Hydrochloride in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Procarbazine 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).

(2) Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200) 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 <*2.03*>. Immerse slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μL each of the sample solution and standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Procarbazine Hydrochloride, previously dried, place in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <*2.50*>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS
= 8.592 mg of $C_{12}H_{19}N_3O.HCl$

Containers and storage Containers—Tight containers.

Procaterol Hydrochloride Hydrate

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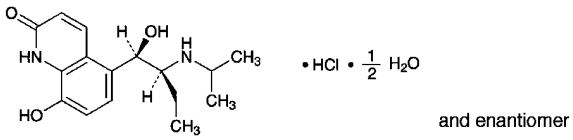


$C_{16}H_{22}N_2O_3.HCl.\frac{1}{2}H_2O$: 335.83
8-Hydroxy-5-{(1*RS*,2*SR*)-1-hydroxy-2-[(1-methylethyl)amino]butyl}quinolin-2(1*H*)-one monohydrochloride hemihydrate
[*62929-91-3*, anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5% of procaterol hydrochloride ($C_{16}H_{22}N_2O_3.HCl$: 326.82), calculated on the anhydrous basis.

Description Procaterol Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Procaterol Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0.

It is gradually colored by light.

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Melting point: about 195°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Procaterol Hydrochloride Hydrate (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procaterol Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3.0 mL of Iron (III) Chloride CS add water to make 50 mL.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Procaterol 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).

(3) Related substances—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sam-

ple solution and standard solution as directed under Liquid Chromatography <2.01> 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 procaterol from the sample solution is not larger than the peak area of procaterol from the standard solution.

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside 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 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of acetic acid (100).

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

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 10 mm.

Time span of measurement: 2.5 times as long as the retention time of procaterol beginning after the solvent peak.

Water <2.48> 2.5 – 3.3% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 30 minutes, cool, add 60 mL of acetic anhydride, and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

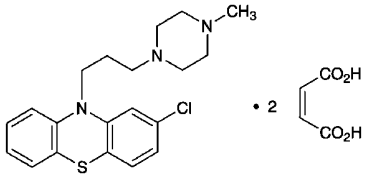
Each mL of 0.1 mol/L perchloric acid VS
= 32.68 mg of $C_{16}H_{22}N_2O_3.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate

プロクロルペラジンマレイン酸塩



$C_{20}H_{24}ClN_3S.2C_4H_4O_4$: 606.09

2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dimaleate

[*84-02-6*]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of $C_{20}H_{24}ClN_3S.2C_4H_4O_4$.

Description Prochlorperazine Maleate occurs as a white to light yellow powder. It is odorless, and has a slightly bitter taste.

It is slightly soluble in acetic acid (100), very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

Identification **(1)** Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

(2) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts <2.60> between 195°C and 198°C (with decomposition).

(3) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue in 10 mL of methanol by warming, and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50°C. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 252°C and 258°C (with decomposition).

(4) To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Prochlorperazine Maleate 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).

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 15.15 mg of $C_{20}H_{24}ClN_3S.2C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$: 606.09).

Method of preparation Prepare as directed under Tablets, with Prochlorperazine Maleate.

Identification **(1)** Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate according to the labeled amount, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium dichromate TS: a green-brown color is produced and changes to brown on standing.

(2) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.08 g of Prochlorperazine Maleate according to the labeled amount, add 15 mL of methanol and 1 mL of dimethylamine, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.08 g of Prochlorperazine Maleate RS in 15 mL of methanol and 1 mL of dimethylamine, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and standard solution show a red-purple color, and has the same *R*f value.

(3) To a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.04 g of Prochlorperazine Maleate according to the labeled amount, add 10 mL of 1 mol/L hydrochloric acid TS and 20 mL of diethyl ether, shake, and centrifuge. Transfer the diethyl ether layer to a separator, wash with 5 mL of 0.05 mol/L sulfuric acid TS, and evaporate on a water bath to dryness. Dissolve the residue in 5 mL of sulfuric acid TS, filter, if necessary, and add 1 to 2 drops of potassium permanganate TS: the red color of the test solution is discharged immediately.

Uniformity of dosage units ⟨*6.02*⟩ 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 Prochlorperazine Maleate Tablets add $3V/5$ mL of a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves until the tablet is disintegrated, and shake vigorously for 10 minutes. Add exactly $V/20$ mL of the internal standard solution, and a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make V mL so that each mL contains about 80 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times V/250$

M_S: Amount (mg) of Prochlorperazine Maleate RS

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Prochlorperazine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Prochlorperazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 9 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank, and determine the absorbances, A_T and A_S, at 255 nm.

Dissolution rate (%) with respect to the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Prochlorperazine Maleate RS
C: Labeled amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Prochlorperazine Maleate Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 8 mg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), and shake vigorously for 10 minutes.

Add exactly 5 mL of the internal standard solution, and add a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prochlorperazine to that of the internal standard.

Amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 2/5$

M_S: Amount (mg) of Prochlorperazine Maleate RS

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside 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 sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of prochlorperazine 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, prochlorperazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

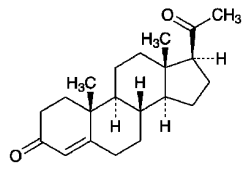
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of prochlorperazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Progesterone

プロゲステロン



$C_{21}H_{30}O_2$: 314.46
Pregn-4-ene-3,20-dione
[*57-83-0*]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{30}O_2$.

Description Progesterone occurs as white crystals or crystalline powder.

It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Progesterone in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Progesterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Progesterone, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Progesterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +184 – +194° (after drying, 0.2 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <*2.60*> 128 – 133°C or 120 – 122°C

Purity Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg each of Progesterone

and Progesterone RS, previously dried, and dissolve each in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solution as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of progesterone ($C_{21}H_{30}O_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Progesterone RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Progesterone Injection

プロゲステロン注射液

Progesterone Injection is an oily solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

Method of preparation Prepare as directed under Injections, with Progesterone.

Description Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

Identification To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, shake well with 1 mL of petroleum benzin, and use the ethanol layer as the sample solution. Separately, dissolve about 5 mg of Progesterone RS in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same *R*f value as the spot from the standard solution.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Measure the specific gravity of Progesterone Injection. Weigh accurately the mass of Progesterone Injection, equivalent to about 1 mL, mix with 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly *V* mL so that each mL contains about 0.5 mg of progesterone ($C_{21}H_{30}O_2$). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Progesterone RS, previously dried in vacuum for 4 hours using phosphorus (V) oxide as the desiccant, dissolve in 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of progesterone to that of the internal standard.

Amount (mg) of progesterone ($C_{21}H_{30}O_2$)
= $M_S \times Q_T/Q_S \times V/20$

M_S: Amount (mg) of Progesterone RS

Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of progesterone is about 6 minutes.

System suitability—

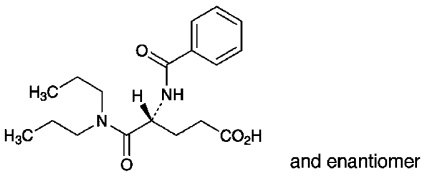
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, progesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Proglumide

プログルミド



$C_{18}H_{26}N_2O_4$: 334.41
(4*RS*)-4-Benzoylamino-*N*,*N*-dipropylglutaramic acid
[*6620-60-6*]

Proglumide, when dried, contains not less than 98.5% of $C_{18}H_{26}N_2O_4$.

Description Proglumide occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

Identification **(1)** Put 0.5 g of Proglumide in a round bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling, open the tube, filter the content to collect crystals separated out, wash the crystals with 50 mL of cold water, and dry at 100°C for 1 hour: the melting point ⟨*2.60*⟩ of the crystals is between 121°C and 124°C.

(2) Determine the infrared absorption spectrum of Proglumide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (225 nm): 384 – 414 (after drying, 4 mg, methanol, 250 mL).

Melting point ⟨*2.60*⟩ 148 – 150°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Proglumide 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 ⟨*1.11*⟩—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the ethanol, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Proglumide in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl acetate, acetic acid (100) and methanol (50:18:5:4) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.10% (1 g, reduced pressure, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

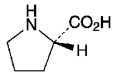
Assay Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.44 mg of $C_{18}H_{26}N_2O_4$

Containers and storage Containers—Well-closed containers.

L-Proline

L-プロリン



$C_5H_9NO_2$: 115.13
(2*S*)-Pyrrolidine-2-carboxylic acid
[*147-85-3*]

L-Proline contains not less than 99.0% and not more than 101.0% of $C_5H_9NO_2$, calculated on the dried basis.

Description L-Proline occurs as white crystals or crystalline powder. It has a slightly sweet taste.

It is very soluble in water and in formic acid, and slightly soluble in ethanol (99.5).

It is deliquescent.

Identification Determine the infrared absorption spectrum of L-Proline as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]^{20}_D$: −84.0 – −86.0° (1 g, calculated on the dried basis, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution of 1.0 g of L-Proline in 10 mL of water is 5.9 to 6.9.

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

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Proline. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Proline. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Proline. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metal ⟨*1.07*⟩—Proceed with 1.0 g of L-Proline 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 ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Proline according to Method 1, 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—Weigh accurately about 0.5 g of L-Proline, and dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount, equivalent to 2.5 mmol, of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-argi-

nine, dissolve them in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the mass percentage of each amino acid, using the mass of amino acid other than proline in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample and standard solution: the amount of each amino acid other than proline is not more than 0.1%.

Operating conditions—

Detector: A visible absorption photometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene (3 μm in particle diameter) (Na type).

Column temperature: A constant temperature of about 57°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, and add 0.1 mL each of caprylic acid.

Mobile phase	A	B	C	D	E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20 mL	4 mL	—	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	—	—
Benzyl alcohol	—	—	—	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Switching of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the above conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in an appropriate amount of water, add 123 mL of acetic acid (100), 401 mL of 1-methoxy-2-propanol and water to make 1000 mL, pass nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, pass nitrogen for 5 minutes, add 81 mg of sodium borohydride, pass nitrogen for 30 minutes, and use this solution as Solution (II). Prepare a mixture with an equal volume of the Solution (I) and (II). (Prepare before use).

Flow rate of mobile phase: 0.20 mL per minute.

Flow rate of reaction regent: 0.24 mL per minute.

System suitability—

System performance: When the test is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak height of each amino acid other than proline in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

(8) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

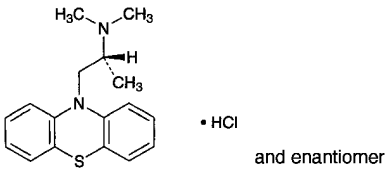
Assay Weigh accurately about 0.12 g of L-Proline, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.51 mg of $C_5H_9NO_2$

Containers and storage Containers—Tight containers.

Promethazine Hydrochloride

プロメタジン塩酸塩



$C_{17}H_{20}N_2S.HCl$: 320.88

(2*RS*)-*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-ylamine monohydrochloride

[*58-33-3*]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of $C_{17}H_{20}N_2S.HCl$.

Description Promethazine Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

A solution of Promethazine Hydrochloride (1 in 25) shows on optical rotation.

Melting point: about 223°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Promethazine Hydrochloride (1 in 100,000) as

directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Promethazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. To 5 mL of the filtrate add dilute nitric acid to make acidic: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> The pH of a solution of Promethazine Hydrochloride (1 in 10) is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting from direct sunlight: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Promethazine 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—Perform the test under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride for thin-layer chromatography in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample 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 methanol and diethylamine (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution corresponding to the spots from the standard solution (2) are not more intense than the spot from the standard solution (2), and any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

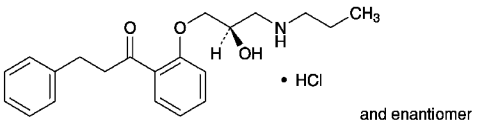
Assay Weigh accurately about 0.5 g of Promethazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.09 mg of $C_{17}H_{20}N_2S.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Propafenone Hydrochloride

プロパフェノン塩酸塩



$C_{21}H_{27}NO_3.HCl$: 377.90
1-{2-[(2*RS*)-2-Hydroxy-3-(propylamino)propyloxy]phenyl}-3-phenylpropan-1-one monohydrochloride
[*34183-22-7*]

Propafenone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{21}H_{27}NO_3.HCl$.

Description Propafenone Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

A solution of Propafenone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification **(1)** Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 3 mL of this solution add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propafenone Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 10 mL of this solution add 1 mL of dilute nitric acid, and filter to separate formed precipitate: the filtrate responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 172 – 175°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Propafenone 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).

(2) Related substances—Dissolve 0.10 g of Propafenone Hydrochloride in 20 mL of the mobile phase in the operating conditions 1, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2.5 mL of this solution, add 2.5 mL of a solution of diphenyl phthalate in methanol (1 in 2000), add the mobile phase in the operating conditions 1 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions 1 and 2. Determine each

peak area of both solutions by the automatic integration method: the area of each peak other than the peak of propafenone from the sample solution is not larger than the peak area of propafenone from the standard solution.

Operating conditions 1—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 39 minutes.

Time span of measurement: Beginning after the solvent peak to the retention time of diphenyl phthalate.

System suitability 1—

System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 1, propafenone and isopropyl benzoate 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 of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

Operating conditions 2—

Detector, column and column temperature: Proceed as directed in the operation conditions 1.

Mobile phase: Dissolve 7.33 g of sodium 1-decanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 700 mL of the filtrate add 700 mL of acetonitrile.

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

Time span of measurement: About 2.5 times as long as the retention time of diphenyl phthalate, beginning after the retention time of diphenyl phthalate.

System suitability 2—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions 2, propafenone and diphenyl phthalate are eluted in this order with the resolution between these peaks being not less than 21.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propafenone Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.90 mg of $C_{21}H_{27}NO_3$.HCl

Containers and storage Containers—Well-closed containers.

Propafenone Hydrochloride Tablets

プロパフェノン塩酸塩錠

Propafenone Hydrochloride Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl: 377.90).

Method of preparation Prepare as directed under Tablets, with Propafenone Hydrochloride.

Identification To a quantity of Propafenone Hydrochloride Tablets, equivalent to 0.3 g of Propafenone Hydrochloride according to the labeled amount, add 60 mL of water, and disintegrate by warming. After cooling, centrifuge, and to 3 mL of the supernatant liquid add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 247 nm and 251 nm, and between 302 nm and 306 nm. Separately, determine the both maximal absorbances, A_1 and A_2, of the solution, the ratio of A_1/A_2 is between 2.30 and 2.55.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propafenone Hydrochloride Tablets add 30 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 6 mg of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl), add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl)
$= M_S \times Q_T/Q_S \times 10/V$

M_S: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Propafenone Hydrochloride Tables is not less than 75%.

Start the test with 1 tablet of Propafenone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Dis-

card 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 67 μg of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 13 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$

M_S: Amount (mg) of propafenone hydrochloride for assay
C: Labeled amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl) in 1 tablet

Assay To a quantity of Propafenone Hydrochloride Tablets, equivalent to 1.5 g of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl), add 70 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, shake well for another 5 minutes, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and centrifuge. Pipet 4 mL of the supernatant liquid, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of propafenone to that of the internal standard.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$.HCl)
$= M_S \times Q_T/Q_S \times 50$

M_S: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propafenone is about 8 minutes.

System suitability—

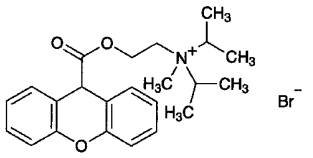
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, propafenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of propafenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propantheline Bromide

プロパンテリン臭化物



$C_{23}H_{30}BrNO_3$: 448.39
N-Methyl-*N*,*N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethylaminium bromide
[*50-34-0*]

Propantheline Bromide, when dried, contains not less than 98.0% and not more than 102.0% of $C_{23}H_{30}BrNO_3$.

Description Propantheline Bromide occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

It is very soluble in water, in ethanol (95), in acetic acid (100) and in chloroform, soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Propantheline Bromide (1 in 50) is between 5.0 and 6.0.

Melting point: about 161°C (with decomposition, after drying).

Identification **(1)** To 5 mL of a solution of Propantheline Bromide (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60°C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 217°C and 222°C.

(2) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) To 5 mL of a solution of Propantheline Bromide (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (1) for bromide.

Purity Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propantheline Bromide in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with

fluorescent indicator for thin-layer chromatography, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

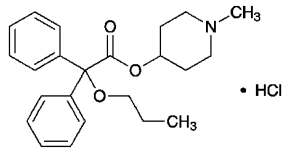
Assay Weigh accurately about 1 g of Propantheline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.84 g of $C_{23}H_{30}BrNO_3$

Containers and storage Containers—Well-closed containers.

Propiverine Hydrochloride

プロピベリン塩酸塩



$C_{23}H_{29}NO_3$.HCl: 403.94
1-Methylpiperidin-4-yl 2,2-diphenyl-2-propoxyacetate monohydrochloride
[*54556-98-8*]

Propiverine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.5% of $C_{23}H_{29}NO_3$.HCl.

Description Propiverine Hydrochloride occurs as white crystals or a white crystalline powder.

It is soluble in water and in ethanol (99.5).

Identification **(1)** Dissolve 50 mg of Propiverine Hydrochloride in 20 mL of water, and add acetonitrile to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Propiverine Hydrochrolide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propiverine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Propiverine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Propiverine Hydrochloride (1 in 100) add 6 mL of ethyl acetate, and add 3 drops of silver nitrate TS: a white precipitate is formed, which does not dissolve on the addition of 0.5 mL of dilute nitric acid and shaking. The precipitate dissolves on the addition of 2 mL of ammonia TS and shaking.

Melting point ⟨*2.60*⟩ 213 – 218°C

Purity **(1)** Sulfate ⟨*1.14*⟩—Perform the test with 0.40 g of Propiverine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Propiverine 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 50 mg of Propiverine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and above mentioned peak from the sample solution is not larger than 1/10 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 1/2 times the peak area of propiverine 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.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

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 propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Propiverine Hydrochloride and Propiverine Hydrachloride RS, both previously dried, and dissolve each in the mobile phase to make exactly 100 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of propiverine from each solution.

Amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Propiverine Hydrochloride RS

System suitability—

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

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

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.21 g of potassium dihydrogen phosphate and 1.51 g of sodium 1-octane sulfonate in 650 mL of water, adjust to pH 3.2 with phosphoric acid, and add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propiverine is about 17 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propiverine Hydrochloride Tablets

プロピベリン塩酸塩錠

Propiverine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl: 403.94).

Method of preparation Prepare as directed under Tablets, with Propiverine Hydrochloride.

Identification Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with 20 mL of water. Add acetonitrile to make 100 mL, centrifuge, and filter the supernatant liquid, if necessary. Determine the absorption spectrum of the supernatant liquid or the filtrate under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Purity Related substances—Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with the mobile phase, add the mobile phase to make 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 7/10 times the peak area of propiverine 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 under Propiverine Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

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 propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propiverine Hydrochloride Tablets add the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 0.1 mg of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Propirevine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

Amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of Propiverine Hydrochloride RS

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 20 minutes of Propiverine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Propiverine Hydrochloride Tablets, withdraw not less than 25 mL of the dissolved solution at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl) according to the labeled amount. Pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, and add the dissolution medium to make exactly 100 mL. Further, pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of propiverine of both solutions.

Dissolution rate (%) with respect to the labeled amount of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Propiverine Hydrochloride RS
C: Labeled amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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: To diluted 0.02 mol/L potassium dihydrogen phosphate TS (1 → 2) add phosphoric acid, and adjust to pH 2.0. To 560 mL of this solution add 440 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propirevine 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, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 4000 and not more than 2.0, respectively.

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

Assay Weigh accurately and powder not less than 20 Propiverine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl), add the mobile phase, shake vigorously, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

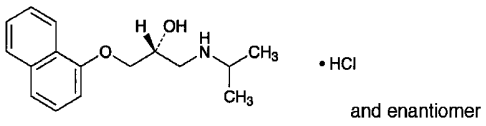
Amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Propiverine Hydrochloride RS

Containers and storage Container—Tight containers.

Propranolol Hydrochloride

プロプラノロール塩酸塩



$C_{16}H_{21}NO_2$.HCl: 295.80
(2*RS*)-1-(1-Methylethyl)amino-3-(naphthalen-1-yloxy)propan-2-ol monohydrochloride
[*318-98-9*]

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{16}H_{21}NO_2$.HCl.

Description Propranolol Hydrochloride occurs as a white, crystalline powder.

It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.

It is gradualy colored to yellowish white to light brown by light.

Identification **(1)** Determine the absorption spectrum of a solution of Propranolol Hydrochloride in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is

5.0 – 6.0.

Melting point ⟨*2.60*⟩ 163 – 166°C

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Propranolol 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 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than propranolol from the sample solution is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 4.6 mm in inside 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 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of propranolol.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 μL of this solution is equivalent to 17 to 33% 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 propranolol is not less than 3000 and not more than 2.0, respectively.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Propranolol Hydrochloride, previously dried, dissolove in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.58 mg of $C_{16}H_{21}NO_2$.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propranolol Hydrochloride Tablets

プロプラノロール塩酸塩錠

Propranolol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl: 295.80).

Method of preparation Prepare as directed under Tablets, with Propranolol Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 288 nm and 292 nm, and between 317 nm and 321 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. 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 20 μg of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of propranolol hydrochloride for assay

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Propranolol Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Propranolol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the

specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 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 sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*.

Dissolution rate (%) with respect to the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of propranolol hydrochloride for assay
C: Labeled amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Propranolol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl), add 60 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Filter, discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*.

Amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= M_S \times A_T/A_S \times 2/5$

M_S: Amount (mg) of propranolol hydrochloride for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propyl Parahydroxybenzoate

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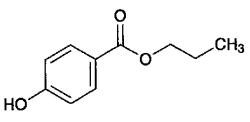


$C_{10}H_{12}O_3$: 180.20
Propyl 4-hydroxybenzoate
[*94-13-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Propyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}O_3$.

◆**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification **(1)** The melting point *⟨2.60⟩* of Propyl Parahydroxybenzoate is between 96°C and 99°C.

◆**(2)** Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry *⟨2.25⟩*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Propyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆**(3)** Heavy metals *⟨1.07⟩*—Dissolve 1.0 g of Propyl Parahydroxybenzoate 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 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 0.10 g of Propyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample 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 Thin-layer Chromatography *⟨2.03⟩*. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with is not more intense than the spot with the standard solution.

Residue on ignition *⟨2.44⟩* Not more than 0.1% (1 g).

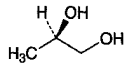
Assay Weigh accurately about 1.0 g of Propyl Parahydroxybenzoate add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate *⟨2.50⟩* the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
$= 180.2$ mg of $C_{10}H_{12}O_3$

◆**Containers and storage** Containers—Well-closed containers.◆

Propylene Glycol

プロピレングリコール



and enantiomer

$C_3H_8O_2$: 76.09
(2*RS*)-Propane-1,2-diol
[*57-55-6*]

Description Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is freely soluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Mix 2 to 3 drops of Propylene Glycol with 0.7 g of triphenylchloromethane, add 1 mL of pyridine, and heat under a reflux condenser on a water bath for 1 hour. After cooling, dissolve the mixture in 20 mL of acetone by warming, shake with 0.02 g of activated charcoal, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the separated crystals, and dry in a desiccator (silica gel) for 4 hours: the crystals melt ⟨*2.60*⟩ between 174°C and 178°C.

(2) Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.035 – 1.040

Purity **(1)** Acidity—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

(2) Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 10.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(4) Heavy metals ⟨*1.07*⟩—Perform the test with 5.0 g of Propylene Glycol according to Method 1. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

(6) Glycerin—Heat 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

Water ⟨*2.48*⟩ Not more than 0.5% (2 g, volumetric titration, direct titration).

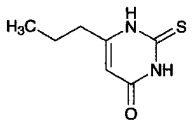
Residue on ignition ⟨*2.44*⟩ Weigh accurately about 20 g of Propylene Glycol in a tared crucible, and heat to boiling. Stop heating, and immediately ignite to burn. Cool, moisten the residue with 0.2 mL of sulfuric acid, and heat strongly with care to constant mass: the mass of the residue is not more than 0.005%.

Distilling range ⟨*2.57*⟩ 184 – 189°C, not less than 95 vol%.

Containers and storage Containers—Tight containers.

Propylthiouracil

プロピルチオウラシル



$C_7H_{10}N_2OS$: 170.23
6-Propyl-2-thiouracil
[*51-52-5*]

Propylthiouracil, when dried, contains not less than 98.0% of $C_7H_{10}N_2OS$.

Description Propylthiouracil occurs as a white powder. It is odorless, and has a bitter taste.

It is sparingly soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification **(1)** Shake well 0.02 g of Propylthiouracil with 7 mL of bromine TS for 1 minute, and heat until the color of bromine TS disappears. Cool, filter, and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

(2) To 5 mL of a hot saturated solution of Propylthiouracil add 2 mL of a solution of sodium pentacyanoammine ferroate (II) *n*-hydrate (1 in 100): a green color develops.

Melting point ⟨*2.60*⟩ 218 – 221°C

Purity **(1)** Sulfate ⟨*1.14*⟩—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 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 0.077%).

(2) Thiourea—Dissolve 0.30 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool, and filter. To 10 mL of the filtrate add 3 mL of ammonia TS, shake well, and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution: Weigh exactly 60 mg of thiourea, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and proceed with 10 mL of this solution in the same manner.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1

mol/L sodium hydroxide VS from a burette, heat to boil, and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small amount of water, and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of $C_7H_{10}N_2OS$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil Tablets

プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$: 170.23).

Method of preparation Prepare as directed under Tablets, with Propylthiouracil.

Identification To a quantity of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil according to the labeled amount, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water, and centrifuge. To the supernatant liquid add acetic acid (31), collect the precipitate produced, recrystallize from water, and dry at 105°C for 1 hour: it melts ⟨*2.60*⟩ between 218°C and 221°C. Proceed with the residue as directed in the Identification under Propylthiouracil.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propylthiouracil Tablets add $3V/4$ mL of 2nd fluid for dissolution test, treat with ultrasonic waves until the tablet is disintegrated, and add 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 0.25 mg of propylthiouracil ($C_7H_{10}N_2OS$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of propylthiouracil for assay

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Propylthiouracil Tablets is not less than 80%.

Start the test with 1 tablet of Propylthiouracil Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of propylthiouracil ($C_7H_{10}N_2OS$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh about 50 mg of propylthiouracil for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 1000 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of propylthiouracil for assay
C: Labeled amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Propylthiouracil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of propylthiouracil ($C_7H_{10}N_2OS$), add 150 mL of 2nd fluid for dissolution test, disperse finely the particles with the aid of ultrasonic waves, and add 2nd fluid for dissolution test to make exactly 200 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propylthiouracil for assay, previously dried at 105°C for 2 hours, and dissolve in 2nd fluid for dissolution test to make exactly 200 mL. Pipet 2 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 absorbance at 274 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of propylthiouracil for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protamine Sulfate

プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to the family *Salmonidae*.

It has a property to bind with heparin.

It binds with not less than 100 Units of heparin per mg, calculated on the dried basis.

Description Protamine Sulfate occurs as a white powder.

It is sparingly soluble in water.

Identification (1) Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10)

and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

(2) Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

(3) An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 1.0 g of Protamine Sulfate in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Absorbance—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance between 260 nm and 280 nm is not more than 0.1.

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 3 hours).

Nitrogen content Weigh accurately about 10 mg of Protamine Sulfate, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N:14.01) is 22.5 – 25.5%, calculated on the dried basis.

Heparin-binding capacity

(i) Sample solution (a)—Weigh accurately about 15 mg of Protamine Sulfate, and dissolve in water to make exactly 100 mL. Repeat this procedure 3 times, and use the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

(ii) Sample solution (b)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 5 mL of water to them, and use these solutions as the sample solutions (b_1), (b_2) and (b_3).

(iii) Sample solution (c)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 20 mL of water to them, and use these solutions as the sample solutions (c_1), (c_2) and (c_3).

(iv) Standard solution—Dissolve Heparin Sodium RS in water to make a solution containing exactly about 20 Units per mL.

(v) Procedure—Transfer exactly 2 mL of the sample solution to a cell for spectrophotometer, add the standard solution dropwise while mixing, and determine the transmittance at 500 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Continue the addition until a sharp change in the transmittance is observed, and note the volume, V mL, of the standard solution added. Repeat this procedure 2 times for each sample solution.

(vi) Calculation—Calculate the amount of heparin bound with 1 mg of the sample by the following formula from the volume of titrant on each sample solution, and calculate the average of 18 results obtained. The assay is not valid unless each relative standard deviation of 6 results obtained from the sample solution (a), sample solution (b) and sample solution (c) is not more than 5%, respectively, and also unless each relative standard deviation of 6 results obtained from 3 sets, (a_1, b_1, c_1), (a_2, b_2, c_2) and (a_3, b_3, c_3) is not more than 5%, respectively.

Amount (heparin Unit) of heparin bound to 1 mg of Protamine Sulfate

$$= S \times V \times 50/M_T \times d$$

S: Amount (heparin Unit) of heparin sodium in 1 mL of the standard solution

M_T: Amount (mg) of the sample, calculated on the dried basis

d: Dilution factor for each sample solution from the sample solution (a)

Sulfate content Weigh accurately about 0.15 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and heat to boil. Add gradually 10 mL of barium chloride TS while boiling, and allow to stand for 1 hour while heating. Filter the precipitate formed, wash the precipitate with warm water several times, and transfer the precipitate into a tared crucible. Dry the precipitate, and incinerate by ignition to constant mass: the amount of sulfate (SO_4) is 16 – 22%, calculated on the dried basis, where 1 g of the residue is equivalent to 0.4117 g of SO_4.

Containers and storage Containers—Tight containers.

Protamine Sulfate Injection

プロタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous solution for injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of Protamine Sulfate. It binds with not less than 100 Units of heparin per mg of the labeled amount.

Method of preparation Prepare as directed under Injections, with Protamine Sulfate.

Description Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

Identification (1) Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

pH <*2.54*> 5.0 – 7.0

Bacterial endotoxins <*4.01*> Less than 6.0 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Protein—Pipet a volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate,

transfer to a Kjeldahl flask, evaporate on a water bath to dryness with the aid of a current of air, determine the nitrogen as directed under Nitrogen Determination ⟨*1.08*⟩, and calculate the amount of protein by converting 0.24 mg of nitrogen (N: 14.01) to 1 mg of protein.

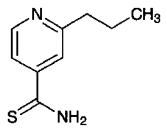
(2) Heparin-binding activity—Proceed the test as directed in the Heparin-binding capacity under Protamine Sulfate, changing the sample solution (a) as below, and determine the amount of heparin bound to 1 mg of protein by dividing by the amount of protein.

(i) Sample solution (a)—Pipet a volume of Protamine Sulfate Injection, equivalent to 15.0 mg of Protamine Sulfate, and add water to make exactly 100 mL. Repeat this procedure two more times, and designate the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

Containers and storage Containers—Hermetic containers.

Prothionamide

プロチオナミド



$C_9H_{12}N_2S$: 180.27
2-Propylpyridine-4-carbothioamide
[*14222-60-7*]

Prothionamide, when dried, contains not less than 98.0% of $C_9H_{12}N_2S$.

Description Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

Identification **(1)** Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

(2) Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt ⟨*2.60*⟩ between 198°C and 203°C (with decomposition).

Melting point ⟨*2.60*⟩ 142 – 145°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acidity—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Prothionamide 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 ⟨*1.11*⟩—Prepare the test solution with 0.6 g of Prothionamide according to Method 3, and perform the test. To the test solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite to burn (not more than 3.3 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ 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 *p*-naphtholbenzein TS). Perform a blank determination.

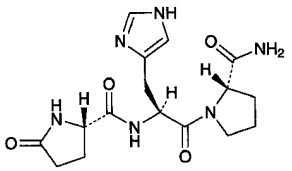
Each mL of 0.1 mol/L perchloric acid VS
= 18.03 mg of $C_9H_{12}N_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protirelin

プロチレリン



$C_{16}H_{22}N_6O_4$: 362.38
5-Oxo-L-prolyl-L-histidyl-L-prolinamide
[*24305-27-9*]

Protirelin contains not less than 98.5% of $C_{16}H_{22}N_6O_4$, calculated on the dehydrated basis.

Description Protirelin occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100).

It is hygroscopic.

Identification **(1)** Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at

110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution show the same color and the same *R*f value as each corresponding spots obtained from the standard solution.

(2) Determine the infrared absorption spectrum of Protirelin, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −66.0 – −69.0° (0.1 g calculated on the dehydrated basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Protirelin in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Protirelin 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 Protirelin in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography, and spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, pyridine and acetic acid (100) (4:2:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plates. Successively spray evenly a solution of sodium carbonate decahydrate (1 in 10) on it: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and heat at 80°C for 5 minutes: no colored spot appears.

Water <2.48> Not more than 5.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.3% (0.2 g).

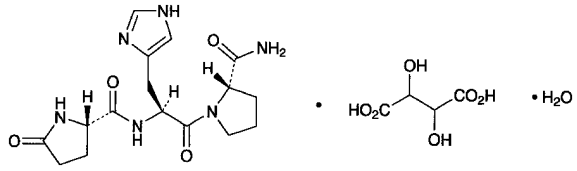
Assay Weigh accurately about 70 mg of Protirelin dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 7.248 mg of $C_{16}H_{22}N_6O_4$

Containers and storage Containers—Tight containers.

Protirelin Tartrate Hydrate

プロチレリン酒石酸塩水和物



$C_{16}H_{22}N_6O_4.C_4H_6O_6.H_2O$: 530.49
5-Oxo-L-prolyl-L-histidyl-L-prolinamide monotartrate monohydrate
[*24305-27-9*, Protirelin]

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate ($C_{16}H_{22}N_6O_4.C_4H_6O_6$: 512.48).

Description Protirelin Tartrate Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: about 187°C (with decomposition).

Identification (1) To 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000) add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

(2) Dissolve 0.03 g of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3) To 0.20 g of Protirelin Tartrate Hydrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride monohydrate and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same *R*f value as the corresponding spot from the standard solution.

(4) A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests <*1.09*> for tartrate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −50.0 – −53.0° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Protirelin Tartrate 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 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Protirelin Tartrate 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 <*1.11*>—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol, and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid, and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat on a water bath to dissolve the residue, use this solution as the test solution, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.60 g of Protirelin Tartrate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography. Spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6:4:1) to a distance of about 10 cm, and dry at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plate. Then, spray evenly a solution of sodium carbonate decahydrate (1 in 10) on the plate: the spots other than the principal spot from the sample solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and dry at 80°C for 5 minutes: no colored spot is obtained.

Water <*2.48*> Not more than 4.5% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

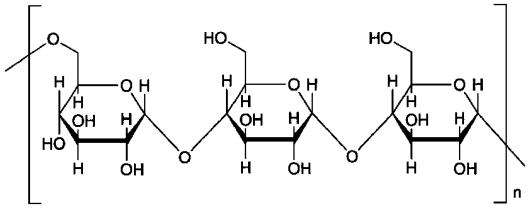
Assay Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 mL of acetic acid (100) by warming, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.25 mg of $C_{16}H_{22}N_6O_4.C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Pullulan

プルラン



$(C_{18}H_{30}O_{15})_n$
Poly[6)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→]
[*9057-02-7*]

Pullulan is a neutral simple polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure of repeated α-1,6 binding of maltotriose composed of three glucoses in α-1,4 binding.

Description Pullulan occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

(2) Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

(3) To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

Viscosity <*2.53*> Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at 30 ± 0.1°C as directed in Method 1: the kinematic viscosity is between 100 and 180 mm^2/s.

pH <*2.54*> Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 4.0 g of Pullulan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Nitrogen—Weigh accurately about 3 g of Pullulan, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is not more than 0.05%. Use 12 mL of sulfuric acid for the decomposition, and add 40 mL of a solution of sodium hydroxide (2 in 5).

(3) Monosaccharide and oligosaccharides—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them gently to each test tube containing 5 mL of a solution

of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with these solutions so obtained as directed under Ultraviolet-visible Spectrophtometry <2.24> using water as a blank, and determine the absorbances at 620 nm, A_T, A_S and A_B: the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (%) of monosaccharide and oligosaccharides
$= (A_T - A_B)/(A_S - A_B) \times 8.2$

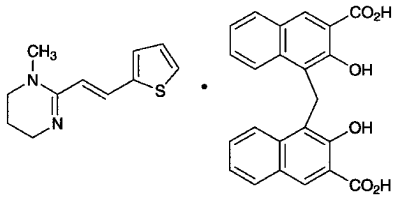
Loss on drying <2.41> Not more than 6.0% (1 g, in vacuum, 90°C, 6 hours).

Residue on ignition <2.44> Not more than 0.3% (2 g).

Containers and storage Containers—Well-closed containers.

Pyrantel Pamoate

ピランテルパモ酸塩



$C_{11}H_{14}N_2S.C_{23}H_{16}O_6$: 594.68
1-Methyl-2-[(1*E*)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydropyrimidine mono[4,4′-methylenebis(3-hydroxy-2-naphthoate)] (1/1)
[*22204-24-6*]

Pyrantel Pamoate, when dried, contains not less than 98.0% of $C_{11}H_{14}N_2S.C_{23}H_{16}O_6$.

Description Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in *N*,*N*-dimethylformamide, very slightly soluble in methanol and in ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: 256 – 264°C (with decomposition).

Identification **(1)** To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of *N*,*N*-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pyrantel Pamoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride <1.03>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed 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) Sulfate <1.14>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Pyrantel Pamoate 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) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 mL of *N*,*N*-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add *N*,*N*-dimethylformamide 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 <2.03>. Spot 5 μL each of the sample 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, 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 spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (*R*f value: about 0.3) from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal

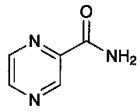
violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of $C_{11}H_{14}N_2S.C_{23}H_{16}O_6$

Containers and storage Containers—Tight containers.

Pyrazinamide

ピラジナミド



$C_5H_5N_3O$: 123.11
Pyrazine-2-carboxamide
[*98-96-4*]

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of $C_5H_5N_3O$.

Description Pyrazinamide occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5) and in acetic anhydride.

Identification **(1)** Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 188 – 193°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pyrazinamide 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 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 20 μL each of the sample 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-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 spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

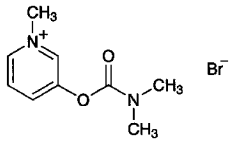
Assay Weigh accurately about 0.1 g of Pyrazinamide, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.31 mg of $C_5H_5N_3O$

Containers and storage Containers—Well-closed containers.

Pyridostigmine Bromide

ピリドスチグミン臭化物



$C_9H_{13}BrN_2O_2$: 261.12
3-Dimethylcarbamoyloxy-1-methylpyridinium bromide
[*101-26-8*]

Pyridostigmine Bromide, when dried, contains not less than 98.5% of $C_9H_{13}BrN_2O_2$.

Description Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Pyridostigmine Bromide (1 in 10) is between 4.0 and 6.0.

It is deliquescent.

Identification **(1)** Dissolve 0.02 g of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a light red precipitate is produced.

(2) To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(3) Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests <*1.09*> for Bromide.

Melting point <*2.60*> 153 – 157°C

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Pyridostigmine Bromide 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 <*1.11*>—Prepare the test solution with 1.0 g

of Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5:4:1) 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 sample solution are not more intense than the spot from the standard solution in color.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

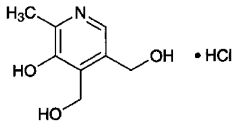
Each mL of 0.1 mol/L perchloric acid VS
= 26.11 mg of $C_9H_{13}BrN_2O_2$

Containers and storage Containers—Hermetic containers.

Pyridoxine Hydrochloride

Vitamin B_6

ピリドキシン塩酸塩



$C_8H_{11}NO_3$.HCl: 205.64
4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol monohydrochloride
[*58-56-0*]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of $C_8H_{11}NO_3$.HCl.

Description Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Pyridoxine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyridoxine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyridoxine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyridoxine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Pyridoxine Hydrochloride 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).

(3) Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water 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 <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-dry ing, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride, and titrate <*2.50*> 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
= 20.56 mg of $C_8H_{11}NO_3$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pyridoxine Hydrochloride Injection

Vitamin B_6 Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl: 205.64).

Method of preparation Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.

It is gradually affected by light.

pH: 3.0 – 6.0

Identification **(1)** To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible spectrophotometry <*2.24*>: it exhibits a maximum between 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.01 g of Pyridoxine Hydrochloride RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate: the spots obtained from the sample solution and the standard solution are blue in color and have the same *R*f value.

Bacterial endotoxins <*4.01*> Less than 3.0 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Pyridoxine Hydrochloride Injection, equivalent to about 20 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl), dilute with water, if necessary, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 1 mL each of the sample solution and standard solution, add 2.0 mL of barbital buffer solution, 9.0 mL of 2-propanol and 2.0 mL of a freshly prepared solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000), shake well, add 2-propanol to make exactly 25 mL, and allow to stand for 90 minutes. Determine the absorbances, A_T and A_S, of the subsequent sample solution and subsequent standard solution, respectively, at 650 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared in the same manner with 1 mL of water, as the blank.

Amount (mg) of pyridoxine hydrochloride ($C_8N_{11}NO_3$.HCl)
$= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of Pyridoxine Hydrochloride RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

Description Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

Identification Ignite Pyroxylin: it burns very rapidly with a luminous flame.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

(2) Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

(3) Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

(4) Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonize the sample, heat strongly at about 500°C for 2 hours, and allow to cool in a desicator (silica gel): the amount of the residue is not more than 0.30%.

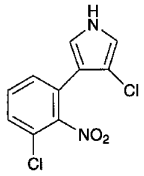
Containers and storage Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from

fire, and preferably in a cold place.

Pyrrolnitrin

ピロールニトリン



$C_{10}H_6Cl_2N_2O_2$: 257.07
3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole
[*1018-71-9*]

Pyrrolnitrin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin ($C_{10}H_6Cl_2N_2O_2$).

Description Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 124 – 128°C

Purity Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin RS, equivalent to about 50 mg (potency) each, and dissolve separetely in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pyrrolnitrin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{10}H_6Cl_2N_2O_2 = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Pyrrolnitrin RS

Internal standard solution—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized 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 acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin 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, pyrrolnitrin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

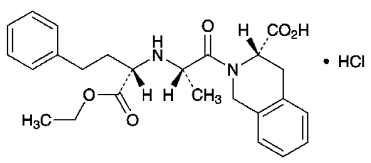
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Quinapril Hydrochloride

キナプリル塩酸塩



$C_{25}H_{30}N_2O_5$.HCl: 474.98
(3*S*)-2-((2*S*)-2-{[(1*S*)-1-Ethoxycarbonyl-3-phenylpropyl]amino}propanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid monohydrochloride
[*82586-55-8*]

Quinapril Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{25}H_{30}N_2O_5$.

HCl, calculated on the anhydrous basis.

Description Quinapril Hydrochloride occurs as a white powder.

It is very soluble in methanol, freely soluble in water and in ethanol (99.5), and soluble in acetic acid (100).

It is deliquescent.

Identification **(1)** Determine the absorption spectrum of a solution of Quinapril Hydrochloride in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinapril Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Quinapril Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +14.4 – +16.0° (0.5 g, calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Quinapril 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 50 mg of Quinapril Hydrochloride in 50 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.5 and about 2.0 to quinapril, obtained from the sample solution are not larger than the peak area of quinapril from the standard solution, respectively, the area of peak other than quinapril and above mentioned peak from the sample solution are not larger than 2/5 times the peak area of quinapril from the standard solution, and the total area of the peaks other than quinapril from the sample solution is not larger than 3 times the peak area of quinapril from the standard solution.

Operating conditions—

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

Column: A stainless steel column 6 mm in inside 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: While keeping the temperature not below 25°C, adjust to pH 2.0 of 0.2 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of quinapril is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of quinapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Confirm that the peak area of quinapril obtained with 10 μL of this solution is equivalent to 7 to 13% 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 quinapril are not less than 5000 and not more than 1.5, respectively.

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

(3) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 1.0% (0.2 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Start to titrate within 3 minutes after dissolving Quinapril Hydrochloride. Weigh accurately about 0.5 g of Quinapril Hydrochloride, dissolve in 70 mL of acetic acid (100), add 4 mL of bismuth nitrate TS, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.50 mg of $C_{25}H_{30}N_2O_5$.HCl

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Quinapril Hydrochloride Tablets

キナプリル塩酸塩錠

Quinapril Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl: 474.98).

Method of preparation Prepare as directed under Tablets, with Quinapril Hydrochloride.

Identification To a quantity of powdered Quinapril Hydrochloride Tablets, equivalent to 20 mg of Quinapril Hydrochloride according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, and centrifuge. To 5 mL of the supernatant liquid add 0.5 mL of dilute hydrochloric acid, and add methanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 256 nm and 260 nm, between 262 nm and 266 nm, and between 269 nm and 273 nm.

Purity To an amount of the supernatant liquid obtained in the Assay add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) so that

each mL contains 0.2 mg of Quinapril Hydrochloride according to the labeled amount, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 0.5 to quinapril obtained from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution, and the area of the peak, having the relative retention time of about 2.0 to quinapril obtained from the sample solution is not larger than the peak area of quinapril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Quinapril 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 the symmetry factor of the peak of quinapril are not less than 5000 and not more than 1.5, respectively.

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

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Quinapril Hydrochloride Tablets add $3V$/5 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablet, shake again for 10 minutes, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V mL so that each mL contains about 0.22 mg of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl), and centrifuge. Pipet 15 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) $= M_S \times Q_T/Q_S \times V/120$

M_S: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Quinapril Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Quinapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V' mL so that each mL contains about 1.2 μg of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of quinapril hydrochloride for assay (separately, determine the water <2.48> in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL. Pipet 2 mL of this solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of quinapril of both solutions.

Dissolution rate (%) with respect to the labeled amount of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) in 1 tablet

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: While keeping the temperature not below 25°C, adjust to pH 2.0 of 0.1 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1500 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of quinapril 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 and the symmetry factor of the peak of quinapril are not less than 2000 and not more than 2.0, respectively.

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

Assay To 20 Quinapril Hydrochloride Tablets add 300 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablets, shake again for 10 minutes, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 500 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to about 6.5 mg of quinapril hydrochlo-

ride ($C_{25}H_{30}N_2O_5$.HCl), add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of quinapril hydrochloride for assay (separately, determine the water *<2.48>* in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Pipet 25 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of quinapril to that of the internal standard.

Amount (mg) of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) in 1 tablet

$= M_S \times Q_T/Q_S \times 1/V \times 25/4$

M_S: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 214 nm).

Column: A stainless steel column 6 mm in inside 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: While keeping the temperature not below 25°C, adjust to pH 2.0 of 0.2 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of quinapril is about 7 minutes.

System suitability—

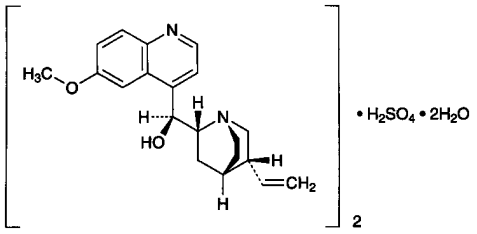
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, quinapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of quinapril to that of the internal standard is not more than 1.0%.

Containers and storage Container—Tight containers.

Quinidine Sulfate Hydrate

キニジン硫酸塩水和物



$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$: 782.94

(9*S*)-6′-Methoxycinchonan-9-ol hemisulfate monohydrate

[*6591-63-5*]

Quinidine Sulfate Hydrate, when dried, contains not less than 98.5% of quinidine sulfate [$(C_{20}H_{24}N_2O_2)_2.H_2SO_4$: 746.91].

Description Quinidine Sulfate Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is freely soluble in ethanol (95) and in boiling water, sparingly soluble in water, and practically insoluble in diethyl ether. Quinidine Sulfate Hydrate, previously dried, is freely soluble in chloroform.

It darkens gradually by light.

Optical rotation $[\alpha]_D^{20}$: +275 – +287° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

Identification **(1)** Dissolve 0.01 g of Quinidine Sulfate Hydrate in 10 mL of water and 2 to 3 drops of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 1000) add 1 to 2 drops of bromine TS, then add 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 100) add 1 mL of silver nitrate TS, stir with a glass rod, and allow to stand for a short interval: a white precipitate is produced, and it dissolves on addition of nitric acid.

(4) Dissolve 0.4 g of Quinidine Sulfate Hydrate in 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests *<1.09>* for sulfate.

pH *<2.54>* Dissolve 1.0 g of Quinidine Sulfate Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity **(1)** Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinidine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at about 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Related substances—Dissolve 20 mg of Quinidine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonine in 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate their amount by the area percentage method: the amount of dihydroquinidine sulfate is not more than 15.0%, and those of quinine sulfate and dihydroquinine sulfate are not more than 1.0%. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

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

Selection of column: Dissolve 0.01 g each of Quinidine Sulfate Hydrate and quinine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with a resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

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

(3) Readily carbonizable substances <1.15>—Take 0.20 g of Quinidine Sulfate Hydrate and perform the test: the solution has no more color than Matching fluid M.

Loss on drying <2.41> Not more than 5.0% (1 g, 130 °C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Quinidine Sulfate Hydrate, previously dried, dissolve in 20 mL of acetic acid (100), and add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

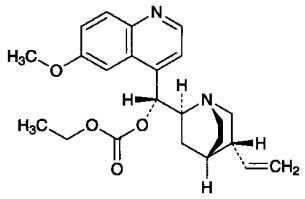
Each mL of 0.1 mol/L perchloric acid VS
= 24.90 mg of $(C_{20}H_{24}N_2O_2)_2.H_2SO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Quinine Ethyl Carbonate

キニーネエチル炭酸エステル



$C_{23}H_{28}N_2O_4$: 396.48

Ethyl (8*S*,9*R*)-6′-methoxycinchonan-9-yl carbonate

[*83-75-0*]

Quinine Ethyl Carbonate contains not less than 98.5% of $C_{23}H_{28}N_2O_4$, calculated on the dehydrated basis.

Description Quinine Ethyl Carbonate occurs as white crystals. It is odorless, and tasteless at first but slowly develops a bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95) and in ethanol (99.5), soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Determine the absorption spectrum of a solution of Quinine Ethyl Carbonate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Ethyl Carbonate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −42.2 – −44.0° (0.5 g, calculated on the dehydrated basis, methanol, 50 mL, 100 mm).

Melting point <2.60> 91 – 95°C

Purity **(1)** Chloride—Dissolve 0.30 g of Quinine Ethyl Carbonate in 10 mL of dilute nitric acid and 20 mL of water. To 5 mL of the solution add 2 to 3 drops of silver nitrate TS: no color develops.

(2) Sulfate <1.14>—Dissolve 1.0 g of Quinine Ethyl Carbonate in 5 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 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Ethyl Carbonate 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 20 mg of Quinine Ethyl Carbonate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of quinine sulfate in the mobile phase to make exactly 100 mL. 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method, and calculate the amount of a main impurity in the sample solution which appears at about 1.2 times of the retention time of quinine ethyl carbonate by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the principal peak and above mentioned peak from the sample solution is not larger than the peak area of quinine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside 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 1.2 g of sodium 1-octanesulfonate in 1000 mL of a mixture of water and methanol (1:1), and adjust to pH 3.5 with diluted phosphoric acid (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of the peak of quinine ethyl carbonate is about 20 minutes.

Selection of column: Dissolve 5 mg each of Quinine Ethyl Carbonate and quinine sulfate in the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinine, dihydroquinine, quinine ethyl carbonate and the main impurity of quinine ethyl carbonate in this order with the resolution between the peaks of quinine and dihydroquinine being not less than 2.7, and between the peaks of quinine and quinine ethyl carbonate being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of quinine obtained from 10 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 2 times as long as the retention time of quinine ethyl carbonate.

Water ⟨*2.48*⟩ Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

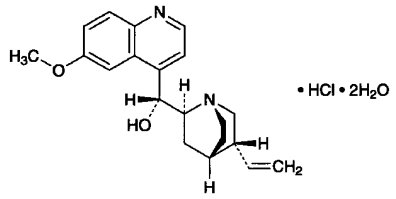
Assay Weigh accurately about 0.3 g of Quinine Ethyl Carbonate, dissolve in 60 mL of acetic acid (100), add 2 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.82 mg of $C_{23}H_{28}N_2O_4$

Containers and storage Containers—Well-closed containers.

Quinine Hydrochloride Hydrate

キニーネ塩酸塩水和物



$C_{20}H_{24}N_2O_2.HCl.2H_2O$: 396.91

(8*S*,9*R*)-6′-Methoxycinchonan-9-ol monohydrochloride dihydrate

[*6119-47-7*]

Quinine Hydrochloride Hydrate, when dried, contains not less than 98.5% of quinine hydrochloride ($C_{20}H_{24}N_2O_2.HCl$: 360.88).

Description Quinine Hydrochloride Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is very soluble in ethanol (99.5), freely soluble in acetic acid (100), in acetic anhydride and in ethanol (95), soluble in water, and practically insoluble in diethyl ether.

It, previously dried, is freely soluble in chloroform.

It gradually changes to brown by light.

Identification **(1)** A solution of Quinine Hydrochloride Hydrate (1 in 50) shows no fluorescence. To 1 mL of the solution add 100 mL of water and 1 drop of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 1000) add 1 to 2 drops of bromine TS and 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 50) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. Collect the precipitate, and add an excess of ammonia TS: it dissolves.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −245 – −255° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Quinine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity **(1)** Sulfate ⟨*1.14*⟩—Perform the test with 1.0 g of Quinine Hydrochloride Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Barium—Dissolve 0.5 g of Quinine hydrochloride Hydrate in 10 mL of water by warming, and add 1 mL of dilute sulfuric acid: no turbidity is produced.

(3) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinine Hydrochloride Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue so obtained is not more than 2.0 mg.

(4) Related substances—Dissolve 20 mg of Quinine Hydrochloride Hydrate in the mobile phase to make exactly 100

mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonidine in 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate the amount of dihydroquinine hydrochloride by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the main peak and the above peaks is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

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

Selection of column: Dissolve 10 mg each of Quinine Hydrochloride and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine, and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine from 50 μL of the standard solution is between 5 mm and 10 mm.

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

(5) Readily carbonizable substances <1.15>—Perform the test with 0.25 g of Quinine Hydrochloride Hydrate. The solution has no more color than Matching Fluid M.

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Quinine Hydrochloride Hydrate, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

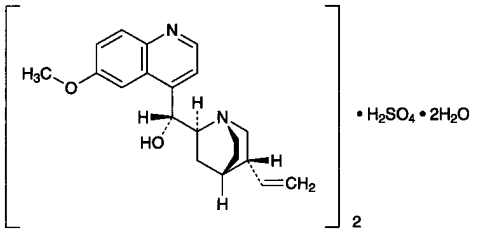
Each mL of 0.1 mol/L perchloric acid VS
= 18.04 mg of $C_{20}H_{24}N_2O_2.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Quinine Sulfate Hydrate

キニーネ硫酸塩水和物



$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$: 782.94

(8*S*,9*R*)-6′-Methoxycinchonan-9-ol hemisulfate monohydrate

[*6119-70-6*]

Quinine Sulfate Hydrate contains not less than 98.5% of quinine sulfate [$(C_{20}H_{24}N_2O_2)_2.H_2SO_4$: 746.91], calculated on the dried basis.

Description Quinine Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a very bitter taste.

It is freely soluble in acetic acid (100), slightly soluble in water, in ethanol (95), in ethanol (99.5) and in chloroform, and practically insoluble in diethyl ether.

It gradually changes to brown by light.

Identification **(1)** Determine the absorption spectrum of a solution of Quinine Sulfate Hydrate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Sulfate Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.4 g of Quinine Sulfate Hydrate add 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: −235 – −245°(after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH <2.54> Shake 2.0 g of Quinine Sulfate Hydrate in 20 mL of freshly boiled and cooled water, and filter: the pH of this filtrate is between 5.5 and 7.0.

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Quinine Sulfate 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) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue is not more than 2.0 mg.

(3) Related substances—Dissolve 20 mg of Quinine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonidine in 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of dihydroquinine sulfate by the area percentage method: it is not more than 5%. The total area of the peaks other than the main peak and the above peak is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

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

Selection of column: Dissolve 0.01 g each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

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

Loss on drying <*2.41*> 3.0% – 5.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Quinine Sulfate Hydrate, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <*2.50*> 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

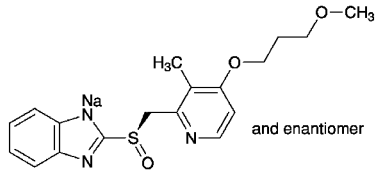
Each mL of 0.1 mol/L perchloric acid VS
= 24.90 mg of $(C_{20}H_{24}N_2O_2)_2.H_2SO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Rabeprazole Sodium

ラベプラゾールナトリウム



$C_{18}H_{20}N_3NaO_3S$: 381.42

Monosodium (*RS*)-2-({[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl}sulfinyl)-1*H*-benzoimidazolide

[*117976-90-6*]

Rabeprazole Sodium contains not less than 98.0% and not more than 101.0% of $C_{18}H_{20}N_3NaO_3S$, calculated on the dried basis.

Description Rabeprazole Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It dissolves in 0.01 mol/L sodium hydroxide TS.

It is hygroscopic.

A solution of Rabeprazole Sodium (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Rabeprazole Sodium in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rabeprazole Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rabeprazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Rabeprazole Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample, or the sample and the RS separately in ethanol (99.5), evaporate the ethanol at 40°C, dry the residues in vacuum at 55°C for 24 hours, and perform the test with the residues.

(3) A solution of Rabeprazole Sodium (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Rabeprazole 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).

(2) Related substances—Dissolve 50 mg of Rabeprazole Sodium in 50 mL of a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following con-

ditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.7 to rabeprazole from the sample solution is not larger than 4/5 times the peak area of rabeprazole from the standard solution, the area of the peak other than rabeprazole and other than the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of rabeprazole from the standard solution, and the total area of the peaks other than rabeprazole from the sample solution is not larger than the peak area of rabeprazole 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.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 100 mL. Confirm that the peak area of rabeprazole obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% 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 rabeprazole are not less than 3000 and not more than 1.5, respectively.

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

(3) Residual solvent—Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours. Take the sample while avoiding moisture absorption.).

Assay Take the sample to be tested while avoiding moisture absorption. Weigh accurately about 0.1 g each of Rabeprazole Sodium and Rabeprazole Sodium RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Rabeprazole Sodium), dissolve each in a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each, then add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of rabeprazole to that of the internal standard.

Amount (mg) of sodium rabeprazole ($C_{18}H_{20}N_3NaO_3S$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Rabeprazole Sodium RS, calculated on the dried basis

Internal standard solution—A solution of 1-amino-2-methylnaphthalene in a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

Column: A stainless steel column 4.6 mm in inside 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 30°C.

Mobile phase: A mixture of methanol and 0.05 mol/L phosphate buffer solution, pH 7.0 (3:2).

Flow rate: Adjust the flow rate so that the retention time of rabeprazole 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, rabeprazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 4, and the symmetry factor of the peak of rabeprazole is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of rabeprazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Freeze-dried Inactivated Tissue Culture Rabies Vaccine

乾燥組織培養不活化狂犬病ワクチン

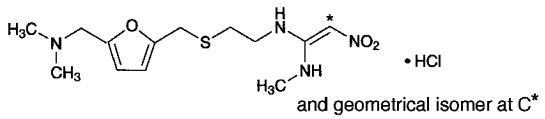
Freeze-dried Inactivated Tissue Culture Rabies Vaccine is a dried preparation containing inactivated rabies virus.

It conforms to the requirements of Freeze-dried Inactivated Tissue Culture Rabies Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Inactivated Tissue Culture Rabies Vaccine becomes a colorless or light yellow-red clear liquid on addition of solvent.

Ranitidine Hydrochloride

ラニチジン塩酸塩



$C_{13}H_{22}N_4O_3S.HCl$: 350.86

(1*EZ*)-*N*-{2-[({5-[(Dimethylamino)methyl]furan-2-yl}methyl)sulfanyl]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine monohydrochloride

[*66357-59-3*]

Ranitidine Hydrochloride, when dried, contains not less than 97.5% and not more than 102.0% of

$C_{13}H_{22}N_4O_3S.HCl$.

Description Ranitidine Hydrochloride occurs as a white to pale yellow, crystalline or fine granular powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

It is gradually colored by light.

Melting point: about 140°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ranitidine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ranitidine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ranitidine Hydrochloride as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Ranitidine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Ranitidine Hydrochloride in 100 mL of water is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—A solution of Ranitidine Hydrochloride (1 in 10) is clear and pale yellow to light yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Ranitidine 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 <*1.11*>—Prepare the test solution with 1.0 g of Ranitidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.22 g of Ranitidine Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 6 mL, 4 mL, 2 mL and 1 mL of the standard solution (1), add to each methanol to make exactly 10 mL, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Separately, dissolve 12.7 mg of ranitidinediamine in methanol to make exactly 10 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography. Separately, spot 10 μL of the sample solution on the plate, then spot 10 μL of the standard solution (6) on the spotted position of the sample solution. Immediately develop the plate with a mixture of ethyl acetate, 2-propanol, ammonia solution (28) and water (25:15:5:1) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand in iodine vapor until the spot from the standard solution (5) appears: the spot obtained from the standard solution (6) is completely separated from the principal spot from the sample solution. The spot having Rf value of about 0.7 from the sample solution is not more intense than the spot from the standard solution (1), the spots other than the principal spot and the spot of Rf value of about 0.7 from the sample solution are not more intense than the spot from the standard solution (2), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.75% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg of Ranitidine Hydrochloride and Ranitidine Hydrochloride RS, previously dried, dissolve each in the mobile phase to make exactly 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ranitidine.

$$\text{Amount (mg) of } C_{13}H_{22}N_4O_3S.HCl = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ranitidine Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 322 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 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 methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 5) (17:3).

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

System suitability—

System performance: Dissolve 20 mg of Ranitidine Hydrochloride and 5 mg of benzalphthalide in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, benzalphthalide and ranitidine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ranitidine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Rape Seed Oil

Oleum Rapae

ナタネ油

Rape Seed Oil is the fixed oil obtained from the seed of *Brassica campestris* Linné subsp. *napus* Hooker filius et Anderson var. *nippo-oleifera* Makino (*Cruciferae*).

Description Rape Seed Oil is a clear, pale yellow, slightly viscous oil. It is odorless or has a slight odor and a mild taste.

It is miscible with diethyl ether and with petroleum diethyl ether. It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25}: 0.906 – 0.920

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 169 – 195

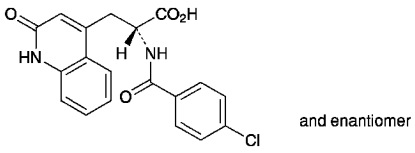
Unsaponifiable matters ⟨*1.13*⟩ Not more than 1.5%.

Iodine value ⟨*1.13*⟩ 95 – 127

Containers and storage Containers—Tight containers.

Rebamipide

レバミピド



and enantiomer

$C_{19}H_{15}ClN_2O_4$: 370.79

(2*RS*)-2-(4-Chlorobenzoylamino)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid

[*90098-04-7*]

Rebamipide, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{15}ClN_2O_4$.

Description Rebamipide occurs as a white crystalline powder, and has a bitter taste.

It is soluble in *N*,*N*-dimethylformamide, very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Rebamipide in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Melting point: about 291°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Rebamipide in methanol (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rebamipide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Rebamipide as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Rebamipide in 40 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 0.40 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Rebamipide 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) Rebamipide *m*-chloro isomer—Dissolve 40 mg of Rebamipide in a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak of rebamipide *m*-chloro isomer, having the relative retention time of about 0.95 with respect to rebamipide, from the sample solution, is not larger than 3/8 times the area of the peak of rebamipide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 4.6 mm in inside 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: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 25 mL. Confirm that the peak area of rebamipide obtained from 10 μL of this solution is equivalent to 15 to 25% of that of rebamipide from 10 μL of the standard solution.

System performance: To 1 mL of the sample solution add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rebamipide are not less than 11,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(4) Related substances—Perform the test with exactly 10 μL each of the sample solution and standard solution obtained in (3) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks of rebamipide *o*-chloro isomer and debenzoylated isomer, having the relative retention times of about 0.5 and about 0.7, respectively, with respect to rebamipide obtained from the sample solution, is not larger than 3/8 times the peak area of rebamipide from the standard solution, the area of each peak other than rebamipide and the peak mentioned above from the sample solution is not larger than 1/4 times the peak area of rebamipide from the standard solution, and the total area of the peaks other than rebamipide from the sample solution is not larger than the peak area of rebamipide from the standard solution. For the calculation, use the peak area of rebamipide *o*-chloro isomer, after multiplying by the response factor, 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: Dissolve 2.44 g of sodium 1-decanesulfonate in 1000 mL of water and to this solution add 1000 mL of methanol and 10 mL of phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 12 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make exactly 50 mL. Confirm that the peak area of rebamipide obtained from 10 μL of this solution is equivalent to 7 to 13% of that of rebamipide from 10 μL of the standard solution.

System performance: Dissolve 20 mg of 4-chlorobenzoate in methanol to make 50 mL. To 5 mL of this solution add 5 mL of the sample solution and a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, rebamipide and 4-chlorobenzoate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Rebamipide, previously dried, dissolve in 60 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide VS until the color of the solution changes from pale yellow to colorless (indicator: 2 drops of phenol red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide VS
= 37.08 mg of $C_{19}H_{15}ClN_2O_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Rebamipide Tablets

レバミピド錠

Rebamipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of rebamipide ($C_{19}H_{15}ClN_2O_4$: 370.79).

Method of preparation Prepare as directed under Tablets, with Rebamipide.

Identification To a quantity of powdered Rebamipide Tablets, equivalent to 30 mg of Rebamipide according to the labeled amount, add 5 mL of a mixture of methanol and ammonia solution (28) (9:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 30 mg of rebamipide for assay in 5 mL of a mixture of methanol and ammonia solution (28) (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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, methanol and formic acid (75:25:2) 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 sample solution has the same *R*f value as the spot from the standard solution.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Rebamipide Tablets add 10 mL of water, shake well for 10 minutes, add exactly 10 mL of the internal standard solution, add 10 mL of *N*,*N*-dimethylformamide, shake well for 5 minutes, and add *N*,*N*-dimethylformamide to make 50 mL. Centrifuge this solution, pipet *V* mL of the supernatant liquid, equivalent to 3 mg of rebamipide ($C_{19}H_{15}ClN_2O_4$), and add 20 mL of *N*,*N*-dimethylformamide and water to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105°C for 2 hours, dissolve in *N*,*N*-dimethylformamide, and add exactly 10 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 50 mL. Pipet 1.5 mL of this solution, add 20 mL of *N*,*N*-dimethylformamide, add water to make 50 mL, and use this solution as the standard

solution. Proceed as directed in the Assay.

Amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= M_S \times Q_T/Q_S \times 3/2V$

M_S: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in *N*,*N*-dimethylformamide (1 in 150).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of diluted disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 (1 in 4), as the dissolution medium, the dissolution rate in 60 minutes of Rebamipide Tablets is not less than 75%.

Start the test with 1 tablet of Rebamipide Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 *V* mL of the subsequent filtrate, add the dissolution medium to make exactly *V′* mL so that each mL contains about 22 μg of rebamipide ($C_{19}H_{15}ClN_2O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of rebamipide for assay, previously dried at 105 °C for 2 hours, and dissolve in *N*,*N*-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank, and determine the absorbances, A_T and A_S, at 326 nm.

Dissolution rate (%) with respect to the labeled amount of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of rebamipide for assay
C: Labeled amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$) in 1 tablet

Assay To 10 Rebamipide Tablets add exactly *V*/5 mL of the internal standard solution and 50 mL of *N*,*N*-dimethylformamide, and disintegrate the tablets with the aid of ultrasonic waves. Shake this solution for 5 minutes, add *N*,*N*-dimethylformamide to make *V* mL so that each mL contains about 10 mg of rebamipide ($C_{19}H_{15}ClN_2O_4$). Centrifuge this solution, and to 5 mL of the supernatant liquid add *N*,*N*-dimethylformamide to make 50 mL. To 2 mL of this solution add 20 mL of *N*,*N*-dimethylformamide and water to make 50 mL. Filter, if necessary, through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105°C for 2 hours, dissolve in *N*,*N*-dimethylformamide, and add exactly 2 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 100 mL. To 2 mL of this solution, add 20 mL of *N*,*N*-dimethylformamide and water to make 50 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of rebamipide to that of the internal standard.

Amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in *N*,*N*-dimethylformamide (1 in 20).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

System suitability—

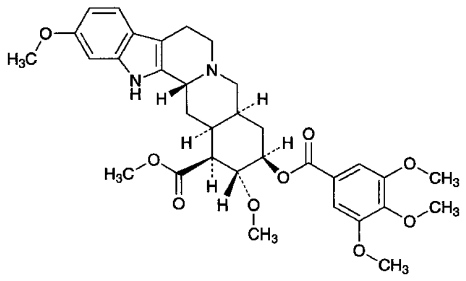
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and rebamipide are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of rebamipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Reserpine

レセルピン



$C_{33}H_{40}N_2O_9$: 608.68
Methyl (3*S*,16*S*,17*R*,18*R*,20*R*)-11,17-dimethoxy-18-(3,4,5-trimethoxybenzoyloxy)yohimban-16-carboxylate [*50-55-5*]

Reserpine, when dried, contains not less than 96.0% of $C_{33}H_{40}N_2O_9$.

Description Reserpine occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in chloroform, slightly soluble in acetonitrile, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is affected by light.

Identification **(1)** To 1 mg of Reserpine add 1 mL of vanillin-hydrochloric acid TS, and warm: a vivid red-purple color develops.

(2) Determine the absorption spectrum of a solution of Reserpine in acetonitrile (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Reserpine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Reserpine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Reserpine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -114 – $-127°$ (after drying, 0.25 g, chloroform, 25 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total area of all peaks other than reserpine peak from the sample solution is not larger than the peak area of reserpine from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 20 minutes.

Time span of measurement: About twice as long as the retention time of reserpine.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add acetonitorile to make exactly 50 mL. Confirm that the peak area of reserpine obtained from 10 μL of this solution is equivalent to 3 to 5% of that from 10 μL of the standard solution.

System performance: Dissolve 0.01 g of Reserpine and 4 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL. When the procedure is run with 20 μL of this solution according to the operating conditions in the Assay, reserpine and butyl parahydroxybenzoate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of reserpine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.2 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 10 mg each of Reserpine and Reserpine RS, previously dried, and dissolve each in acetonitrile to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of reserpine to that of the internal standard.

$$\text{Amount (mg) of } C_{33}H_{40}N_2O_9 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Reserpine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of reserpine 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, reserpine and the internal standard 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of reserpine to that of the internal standard is not more than 2.0%.

Containers and Storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Injection

レセルピン注射液

Reserpine Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation Prepare as directed under Injections, with Reserpine.

Description Reserpine Injection is a clear, colorless or pale yellow liquid.

pH: 2.5 – 4.0

Identification Measure a volume of Reserpine Injection, equivalent to 1.5 mg of Reserpine according to the labeled amount, add 10 mL of diethyl ether, shake for 10 minutes, and take the aqueous layer. If necessary, add 10 mL of diethyl ether to the aqueous layer, and shake for 10 minutes to repeat the process. To the aqueous layer add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 265 nm and 269 nm.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Reserpine Injection, equivalent to about 4 mg of reserpine ($C_{33}H_{40}N_2O_9$). Separately, weigh accurately about 4 mg of Reserpine RS, previously dried in vacuum at 60°C for 3 hours. Transfer them to separate separator, add 10 mL each of water and 5 mL each of ammonia TS, and extract with one 20-mL portion of chloroform, then with three 10-mL portions of chloroform with shaking vigorously. Combine the chloroform extracts, wash with two 50-mL portions of diluted hydrochloric acid (1 in 1000), and combine the washings. Then wash the chloroform extract with two 50-mL portions of a solution of sodium hydrogen carbonate (1 in 100), and combine the all washings. Extract the combined washing with two 10-mL portions of chloroform, and combine the washings with the former chloroform extract. Transfer the chloroform solution to a 100-mL volumetric flask through a pledget of absorbent cotton previously wetted with chloroform, wash with a small amount of chloroform, dilute with chloroform to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Amount (mg) of reserpine } (C_{33}H_{40}N_2O_9) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Reserpine RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

0.1% Reserpine Powder

Reserpine Powder

レセルピン散 **0.1%**

0.1% Reserpine Powder contains not less than 0.09% and not more than 0.11% of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation

Reserpine	1 g
Lactose Hydrate	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Identification To 0.4 g of 0.1% Reserpine Powder add 20 mL of acetonitrile, shake for 30 minutes, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of 0.1% Reserpine Powder, equivalent to about 0.5 mg of reserpine ($C_{33}H_{40}N_2O_9$), disperse in 12 mL of water, add exactly 10 mL of the internal standard solution and 10 mL of acetonitrile, and dissolve by warming at 50°C for 15 minutes, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

$$\text{Amount (mg) of reserpine } (C_{33}H_{40}N_2O_9) = M_S \times Q_T/Q_S \times 1/20$$

M_S: Amount (mg) of Reserpine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Tablets

レセルピン錠

Reserpine Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation Prepare as directed under Tablets, with Reserpine.

Identification Take a portion of powdered Reserpine Tablets, equivalent to 0.4 mg of Reserpine according to the labeled amount, add 20 mL of acetonitrile, shake for 30 minute, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure without exposure to daylight, using light-resistant vessels. To 1 tablet of Reserpine Tablets add 2 mL of water, disintegrate by warming at 50°C for 15 minutes while shaking. After cooling, add exactly 2 mL of the internal standard solution per 0.1 mg of reserpine ($C_{33}H_{40}N_2O_9$), add 2 mL of acetonitrile, warm at 50°C for 15 minutes while shaking, and after cooling, add water to make 10 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

Amount (mg) of reserpine ($C_{33}H_{40}N_2O_9$)
$= M_S \times Q_T/Q_S \times C/10$

M_S: Amount (mg) of Reserpine RS
C: Labeled amount (mg) of reserpine in 1 tablet

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of a solution of polysorbate 80 (1 in 20,000) in diluted dilute acetic acid (1 in 200) as the dissolution medium, the dissolution rate in 30 minutes of Reserpine Tablets is not less than 70%.

Start the test with 1 tablet of Reserpine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter through a filter laminated with polyester fibers, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Reserpine RS at 60°C in vacuum for 3 hours, weigh accurately an amount 100 times the labeled amount of Reserpine Tablets, dissolve in 1 mL of chloroform and 80 mL of ethanol (95), and add the dissolution medium to make exactly 200 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, transfer to glass-stoppered brown test tubes T and S, respectively, add exactly 5 mL each of ethanol (99.5), shake well, add exactly 1 mL each of diluted vanadium (V) oxide TS (1 in 2), shake vigorously, and allow to stand for 30 minutes. Perform the test with these solutions as directed under Fluorometry ⟨*2.22*⟩, and determine the intensity of fluorescence, F_T and F_S, at the wavelength of excitation at 400 nm and at the wavelength of fluorescence at 500 nm.

Dissolution rate (%) with respect to the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$)
$= M_S \times F_T/F_S \times 1/C$

M_S: Amount (mg) of Reserpine RS
C: Labeled amount (mg) of reserpine ($C_{33}H_{40}N_2O_9$) in 1 tablet

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately and powder not less than 20 Reserpine Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.5 mg of reserpine ($C_{33}H_{40}N_2O_9$), add 3 mL of water, and warm at 50°C for 15 minutes while shaking. After cooling, add exactly 10 mL of the internal standard solution, 10 mL of acetonitrile and warm at 50°C for 15 minutes while shaking. After cooling, add water to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

Amount (mg) of reserpine ($C_{33}H_{40}NO_9$)
$= M_S \times Q_T/Q_S \times 1/20$

M_S: Amount (mg) of Reserpine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

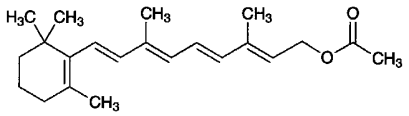
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Retinol Acetate

Vitamin A Acetate

レチノール酢酸エステル



$C_{22}H_{32}O_2$: 328.49
(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl acetate
[*127-47-9*]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil.

It contains not less than 2,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

It is freely soluble in petroleum ether, soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Acetate and Retinol Acetate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a

distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and *R*f value with the blue spot from the standard solution.

Purity **(1)** Acid value <*1.13*>—Take exactly 5.0 g of Retinol Acetate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Acetate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate <*2.50*> this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 mEq/kg.

$$\text{Amount (mEq/kg) of peroxide} = V/M \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
M: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <*2.55*>.

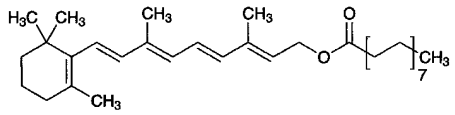
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Retinol Palmitate

Vitamin A Palmitate

レチノールパルミチン酸エステル



$C_{36}H_{60}O_2$: 524.86
(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl palmitate
[*79-81-2*]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil.

It contains not less than 1,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

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

It is decomposed by air and by light.

Identification Dissolve Retinol Palmitate and Retinol Palmitate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and *R*f value with the blue spot from the standard solution.

Purity **(1)** Acid value <*1.13*>—Take exactly 5.0 g of Retinol Palmitate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Palmitate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate <*2.50*> this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 mEq/kg.

$$\text{Amount (mEq/kg) of peroxide} = V/M \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS
M: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay <*2.55*>.

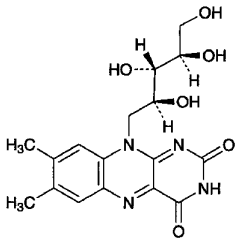
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Riboflavin

Vitamin B_2

リボフラビン



$C_{17}H_{20}N_4O_6$: 376.36
7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione
[*83-88-5*]

Riboflavin, when dried, contains not less than 98.0% of $C_{17}H_{20}N_4O_6$.

Description Riboflavin occurs as yellow to orange-yellow crystals. It has a slight odor.

It is very slightly soluble in water, practically insoluble in ethanol (95), in acetic acid (100), and in diethyl ether.

It dissolves in sodium hydroxide TS.

A saturated solution of Riboflavin is neutral.

It is decomposed by light.

Melting point: about 290°C (with decomposition).

Identification **(1)** A solution of Riboflavin (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin in phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rivoflavin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -128 – $-142°$ Weigh accurately about 0.1 g of dried Riboflavin, dissolve in exactly 4 mL of dilute sodium hydroxide TS, add 10 mL of freshly boiled and cooled water, add exactly 4 mL of aldehyde-free ethanol while shaking, add freshly boiled and cooled water to make exactly 20 mL, and determine the rotation in a 100-mm cell within 30 minutes after preparing the solution.

Purity Lumiflavin—Shake 25 mg of Riboflavin with 10 mL of ethanol-free chloroform for 5 minutes, and filter: the filtrate has no more color than the following control solution.

Control solution: To 2.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

Loss on drying <*2.41*> Not more than 1.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 15 mg of Riboflavin, previously dried, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the sample solution. Dry Riboflavin RS at 105°C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank, and determine the absorbances, A_T and A_S, at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, A_T' and A_S', of the solutions.

$$\text{Amount (mg) of } C_{17}H_{20}N_4O_6 = M_S \times (A_T - A_T')/(A_S - A_S')$$

M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Riboflavin Powder

Vitamin B_2 Powder

リボフラビン散

Riboflavin Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$: 376.36).

Method of preparation Prepare as directed under Granules or Powders, with Riboflavin.

Identification Shake a portion of Riboflavin Powder, equivalent to 1 mg of Riboflavin according to the labeled amount, with 100 mL of water, filter, and proceed with the filtrate as directed in the Identification (1) and (2) under Riboflavin.

Purity Rancidity—Riboflavin Powder is free from any unpleasant or rancid odor or taste.

Assay The procedure should be performed under protection from direct sunlight and in light-resistant vessels. Weigh accurately Riboflavin Powder equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add 800 mL of diluted acetic acid (100) (1 in 400), and extract by warming for 30 minutes with occasional shaking. Cool, dilute with water to make exactly 1000 mL, and filter through a glass filter (G4). Use this filtrate as the sample solution, and proceed as directed in the Assay under Riboflavin.

$$\text{Amount (mg) of riboflavin } (C_{17}H_{20}N_4O_6) = M_S \times (A_T - A_T')/(A_S - A_S')$$

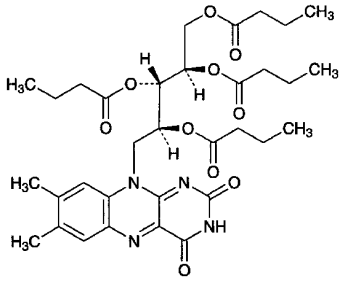
M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Riboflavin Butyrate

リボフラビン酪酸エステル



$C_{33}H_{44}N_4O_{10}$: 656.72
(2*R*,3*S*,4*S*)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)pentan-1,2,3,4-tetrayl tetrabutanoate
[*752-56-7*]

Riboflavin Butyrate, when dried, contains not less than 98.5% of $C_{33}H_{44}N_4O_{10}$.

Description Riboflavin Butyrate occurs as orange-yellow crystals or crystalline powder. It has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in diethyl ether, and practically insoluble in water.

It is decomposed by light.

Identification **(1)** A solution of Riboflavin Butyrate in ethanol (95) (1 in 100,000) shows a light yellow-green color with a strong yellowish green fluorescence. To the solution add dilute hydrochloric acid or sodium hydroxide TS: the fluorescence disappears.

(2) Dissolve 0.01 g of Riboflavin Butyrate in 5 mL of ethanol (95), add 2 mL of a mixture of a solution of hydroxylammonium chloride (3 in 20) and a solution of sodium hydroxide (3 in 20) (1:1), and shake well. To this solution add 0.8 mL of hydrochloric acid and 0.5 mL of iron (III) chloride TS, and add 8 mL of ethanol (95): a deep red-brown color develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 146 – 150°C

Purity **(1)** Chloride—Dissolve 2.0 g of Riboflavin Butyrate in 10 mL of methanol, and add 24 mL of dilute nitric acid and water to make 100 mL. After shaking well, allow to stand for 10 minutes, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 25 mL of the sample solution add water to make 50 mL, then add 1 mL of silver nitrate TS, and allow to stand for 5 minutes: the turbidity of the solution is not thicker than that of the following control solution.

Control solution: To 25 mL of the sample solution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter. Wash the precipitate with four 5-mL portions of water, and combine the washings with the filtrate. To this solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, add 1 mL of water, and mix (not more than 0.021%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Riboflavin Butyrate 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) Free acid—To 1.0 g of Riboflavin Butyrate add 50 mL of freshly boiled and cooled water, shake, and filter. To 25 mL of the filtrate add 0.50 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution shows a red color.

(4) Related substances—Dissolve 0.10 g of Riboflavin Butyrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform 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 <*2.03*>. Spot 10 μL each of the sample 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 and 2-propanol (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 40 mg of Riboflavin Butyrate, previously dried, dissolve in ethanol (95) to make exactly 500 mL, and pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105°C for 2 hours, dissolve in 150 mL of diluted acetic acid (100) (2 in 75) by warming, and after cooling, add water to make exactly 500 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 445 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{33}H_{44}N_4O_{10} = M_S \times A_T/A_S \times 1/2 \times 1.745$$

M_S: Amount (mg) of Riboflavin RS

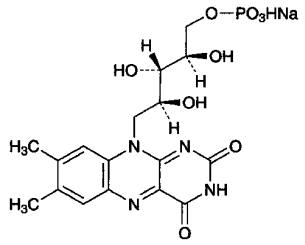
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Riboflavin Sodium Phosphate

Riboflavin Phosphate
Vitamin B_2 Phosphate Ester

リボフラビンリン酸エステルナトリウム



$C_{17}H_{20}N_4NaO_9P$: 478.33
Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl monohydrogenphosphate
[*130-40-5*]

Riboflavin Sodium Phosphate contains not less than 92% of $C_{17}H_{20}N_4NaO_9P$, calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate is a yellow to orange-yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in water, and practically insoluble in ethanol (95), in chloroform and in diethyl ether.

It is decomposed on exposure to light.

It is very hygroscopic.

Identification **(1)** A solution of Riboflavin Sodium Phosphate (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin Sodium Phosphate in phosphate buffer solution, pH 7.0, (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 0.05 g of Riboflavin Sodium Phosphate add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. Boil the residue with 10 mL of nitric acid (1 in 50) for 5 minutes, after cooling, neutralize this solution with ammonia TS, and filter, if necessary: the solution responds to the Qualitative Tests <*1.09*> for sodium salt and phosphate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +38 – +43° (0.3 g, calculated on the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH <*2.54*> Dissolve 0.20 g of Riboflavin Sodium Phosphate in 20 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Riboflavin Sodium Phosphate in 10 mL of water: the solution is clear and yellow to orange-yellow in color.

(2) Lumiflavin—To 35 mg of Riboflavin Sodium Phosphate add 10 mL of ethanol-free chloroform, and shake for 5 minutes, then filter: the filtrate has no more color than the control solution.

Control solution: To 3.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(3) Free phosphoric acid—Weigh accurately about 0.4 g of Riboflavin Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 5 mL each of the sample solution and Phosphoric Acid Standard Solution, transfer to separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS to each of these flasks, mix, and add water to make 25 mL. Allow to stand for 30 minutes at 20 ± 1°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of water in the same manner as a blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and standard phosphoric acid solution at 740 nm: the free phosphoric acid content is not more than 1.5%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of Riboflavin Sodium Phosphate calculated on the anhydrous basis

Water <*2.48*> Place 25 mL of a mixture of methanol for Karl Fischer method and ethylene glycol for Karl Fischer method (1:1) in a dry flask for titration, and titrate with water determination TS to the end point. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, place quickly into the flask, add a known excess volume of Karl Fischer TS, mix for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To about 0.1 g of Riboflavin Sodium Phosphate, accurately weighed, dissolve in diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, then pipet 10 mL of this solution, and add diluted acetic acid (100) (1 in 500) to make exactly 50 mL. Use this solution as the sample solution. Separately, dry Riboflavin RS at 105 °C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank, and determine the absorbances, A_T and A_S, at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, A_T' and A_S', of the solutions.

Amount (mg) of $C_{17}H_{20}N_4NaO_9P$
$= M_S \times (A_T - A_T') / (A_S - A_S') \times 5 \times 1.271$

M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate Injection

Riboflavin Phosphate Injection
Vitamin B_2 Phosphate Ester Injection

リボフラビンリン酸エステルナトリウム注射液

Riboflavin Sodium Phosphate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 120.0% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$: 376.36).

The concentration of Riboflavin Sodium Phosphate Injection should be stated as the amount of riboflavin ($C_{17}H_{20}N_4O_6$).

Method of preparation Prepare as directed under Injections, with Riboflavin Sodium Phosphate.

Description Riboflavin Sodium Phosphate Injection is a clear, yellow to orange-yellow liquid.

pH: 5.0 – 7.0

Identification **(1)** To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 1 mg of Riboflavin according to the labeled amount, add water to make 100 mL, and proceed with this solution as directed in the Identification (1) and (2) under Riboflavin Sodium Phosphate.

(2) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 0.05 g of Riboflavin according to the labeled amount, and evaporate on a water bath to dryness. Proceed with this residue as directed in the Identification (4) under Riboflavin Sodium Phosphate.

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an accurately measured volume of Riboflavin Sodium Phosphate Injection, equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Riboflavin Sodium Phosphate.

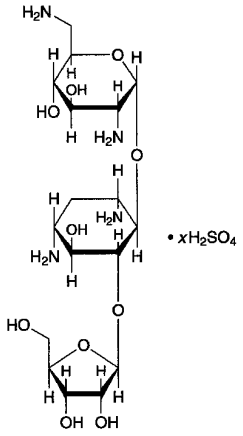
Amount (mg) of Riboflavin ($C_{17}H_{20}N_4O_6$)
$= M_S \times (A_T - A_T') / (A_S - A_S')$

M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Ribostamycin Sulfate

リボスタマイシン硫酸塩



$C_{17}H_{34}N_4O_{10}.xH_2SO_4$
2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-[β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine sulfate
[*53797-35-6*]

Ribostamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces ribosidificus*.

It contains not less than 680 μg (potency) and not more than 780 μg (potency) per mg, calculated on the dried basis. The potency of Ribostamycin Sulfate is expressed as mass (potency) of ribostamycin ($C_{17}H_{34}N_4O_{10}$: 454.47).

Description Ribostamycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Dissolve 20 mg of Ribostamycin Sulfate in 2 mL of phosphate buffer solution, pH 6.0, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 0.12 g each of Ribostamycin Sulfate and Ribostamycin Sulfate RS in 20 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) 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 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same *R*f value.

(3) To 2 mL of a solution of Ribostamycin Sulfate (1 in

5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +42 – +49° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Ribostamycin 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 Ribostamycin Sulfate in 5 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ribostamycin 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).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.12 g of Ribostamycin Sulfate in water to make exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample 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 ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) 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 at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Ribostamycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), 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 at 5 to 15°C and use within 20 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 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 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 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rice Starch

Amylum Oryzae

コメデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Rice Starch consists of the starch granules obtained from the caryopsis of *Oryza sativa* Linné (*Gramineae*).

◆**Description** Rice Starch occurs as a white mass or powder.

It is practically insoluble in water and in ethanol (99.5).◆

Identification **(1)** Examined under a microscope ⟨*5.01*⟩ using a mixture of water and glycerin (1:1), Rice Starch presents polyhedral, simple grains 1 – 10 μm, mostly 4 – 6 μm, in size. These simple grains often gather in ellipsoidal, compound grains 50 – 100 μm in diameter. The granules have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Rice Starch add 50 mL of water, boil for 1 minute, and allow to cool: a thin, cloudy mucilage is formed.

(3) To 1 mL of the mucilage obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to dark-blue color is produced which disappears on heating.

pH ⟨*2.54*⟩ To 5.0 g of Rice Starch add 25 mL of freshly boiled and cooled water, and mix gently for 1 minute to achieve suspension. Allow to stand for 15 minutes: the pH of the solution is 5.0 to 8.0.

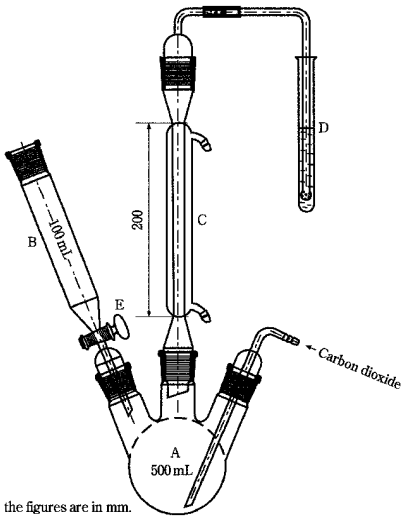
Purity **(1)** Iron—To 1.5 g of Rice Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use this solution as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Add ammonia solution (28) to these solutions until the color of a litmus paper to change from red to blue, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Rice Starch add 50 mL of water, shake for 5 minutes, and centrifuge. To 30 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes in the dark. Add 1 mL of starch TS, and titrate ⟨*2.50*⟩ with 0.002 mol/L sodium thiosulfate VS until the starch-iodine color disappears. Perform a blank determi-

nation in the same manner, and make any necessary correction. Not more than 1.4 mL of 0.002 mol/L sodium thiosulfate VS is required (not more than 20 ppm, calculated as H_2O_2).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Boiling flask (500 mL)
B: Funnel (100 mL)
C: Condenser
D: Test-tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Rice Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat on a water-bath for 15 minutes and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of the sample

V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

◆**(4)** Foreign matter—Under a microscope <5.01>, Rice Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.◆

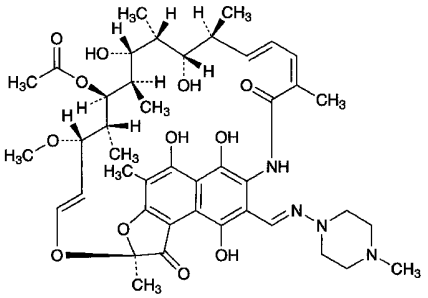
Loss on drying <2.41> Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers.◆

Rifampicin

リファンピシン



$C_{43}H_{58}N_4O_{12}$: 822.94

(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1-yliminomethyl)-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-21-yl acetate

[*13292-46-1*]

Rifampicin is a derivative of a substance having antibacterial activity produced by the growth of *Streptomyces mediterranei*.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Rifampicin is expressed as mass (potency) of rifampicin ($C_{43}H_{58}N_4O_{12}$).

Description Rifampicin occurs as orange-red to red-brown, crystals or crystalline powder.

It is slightly soluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification **(1)** To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rifampicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rifampicin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and

compare the spectrum with the Reference Spectrum or the spectrum of Rifampicin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Rifampicin 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 <*1.11*>—Prepare the test solution with 1.0 g of Rifampicin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Perform the test immediately after preparing of the sample and standard solutions. Dissolve 0.10 g of Rifampicin in 50 mL of acetonitrile, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 1 mL of the sample stock solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak appeared at the relative retention time of about 0.7 with respect to rifampicin from the sample solution is not larger than 1.5 times the peak area of rifampicin from the standard solution, the area of the peak other than rifampicin and the peak mentioned above from the sample solution is not larger than the peak area of rifampicin from the standard solution, and the total area of the peaks other than rifampicin and the peak mentioned above from the sample solution is not larger than 3.5 times the peak area of rifampicin 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.

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

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add citric acid-phosphate-acetonitrile TS to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0%.

Loss on drying <*2.41*> Not more than 2.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Rifampicin and Rifampicin RS, equivalent to about 40 mg (potency), and dissolve each in acetonitrile to make exactly 200 mL. Pipet 10 mL each of these solutions, add citric acid-phosphate-acetonitrile TS to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of rifampicin.

$$\text{Amount [}\mu\text{g (potency)] of } C_{43}H_{58}N_4O_{12} = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Rifampicin RS

Operating conditions—

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

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in 1000 mL of a mixture of water, acetonitrile and phosphate buffer solution, pH 3.1 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 8 minutes.

System suitability—

System performance: To 5 mL of a solution of Rifampicin in acetonitrile (1 in 5000) add 1 mL of a solution of butyl parahydroxybenzoate in acetonitrile (1 in 5000) and citric acid-phosphate-acetonitrile TS to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Rifampicin Capsules

リファンピシンカプセル

Rifampicin Capsules contain not less than 93.0% and not more than 105.0% of rifampicin ($C_{43}H_{58}N_4O_{12}$: 822.94).

Method of preparation Prepare as directed under Capsules, with Rifampicin.

Identification Take out the contert of Rifampicin Capsules, mix well, and powder, if necessary. Dissolve an amount of the content, equivalent to 20 mg (potency) of Rifampicin according to the labeled amount, in methanol to make 100 mL, and filter. To 5 mL of the filtrate add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 234 nm and 238 nm, between 252 nm and 256 nm, between 331 nm and 335 nm, and between 472 nm and 476 nm.

Purity Related substances—Perform the test quickly after

the sample solution and standard solution are prepared. Open the capsules of not less than 20 Rifampicin Capsules, carefully take out the content, weigh accurately, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg (potency) of Rifampicin according to the labeled amount, and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 20 mg (potency), and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, and add the mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Pipet 1 mL of this solution, add the mixture of acetonitrile and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the amount of the peaks of quinone substance and *N*-oxide substance, having the relative retention time of about 0.5 and about 1.2 with respect to rifampicin, obtained from the sample solution are not more than 4.0% and not more than 1.5%, respectively. The amount of the peak other than the peaks mentioned above is not more than 1.0%, and the total amount of these related substances is not more than 2.0%. For these calculations, use the areas of the peaks of the quinone substance and *N*-oxide substance after multiplying by their relative response factors, 1.24 and 1.16, respectively.

$$\text{Amount (mg) of quinone substance} = M_S/M_T \times A_{Ta}/A_S \times 2.48$$

$$\text{Amount (mg) of } N\text{-oxide substance} = M_S/M_T \times A_{Tb}/A_S \times 2.32$$

Each amount (mg) of related substances other than quinone and *N*-oxide substances = $M_S/M_T \times A_{Ti}/A_S \times 2$

M_S: Amount [mg (potency)] of Rifampicin RS
M_T: Amount [mg (potency)] of sample
A_S: Peak area of the standard solution
A_{Ta}: Peak area of quinone substance
A_{Tb}: Peak area of *N*-oxide substance
A_{Ti}: Each peak area of related substances other than quinone and *N*-oxide substances

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 25°C.

Mobile phase: Dissolve 2.1 g of sodium perchlorate, 6.5 g of citric acid monohydrate and 2.3 g of potassium dihydrogen phosphate in 1100 mL of water, and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of rifampicin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of rifampicin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% 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 rifampicin is not less than 2500 and not more than 4.0, respectively.

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

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Rifampicin Capsules is not less than 80%.

Start the test with 1 capsule of Rifampicin Capsules, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 17 μg (potency) of rifampicin ($C_{43}H_{58}N_4O_{12}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg (potency) of Rifampicin RS, dissolve in 5 mL of methanol, and add water to make exactly 100 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 334 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of rifampicin } (C_{43}H_{58}N_4O_{12}) = M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount [mg (potency)] of Rifampicin RS
C: Labeled amount [mg (potency)] of rifampicin ($C_{43}H_{58}N_4O_{12}$) in 1 capsule

Assay Open the capsules of not less than 20 Rifampicin Capsules, take out the content, weigh accurately the mass of the content, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Rifampicin, dissolve in a mixture of acetonitrile and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, and add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 30 mg (potency), dissolve in 20 mL of a mixture of acetonitrile and methanol (1:1), and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the solution prepared by dissolving 2.1 g of citric acid monohydrate,

27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of the mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of rifampicin.

Amount [mg (potency)] of rifampicin ($C_{43}H_{58}N_4O_{12}$)
$= M_S \times A_T/A_S \times 5/2$

M_S: Amount [mg (potency)] of Rifampicin RS

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Rifampicin.

System suitability—

System performance: Dissolve 30 mg (potency) of Rifampicin RS in 20 mL of the mixture of acetonitrile and methanol (1:1), and add acetonitrile to make 100 mL. To 5 mL of this solution add 2 mL of a solution of butyl parahydroxybenzoate in the mixture of acetonitrile and methanol (1:1) (1 in 5000), then add the solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1) to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ringer's Solution

リンゲル液

Ringer's Solution is an aqueous solution for injection.

It contains not less than 0.53 w/v% and not more than 0.58 w/v% of chlorine [as (Cl: 35.45)], and not less than 0.030 w/v% and not more than 0.036 w/v% of calcium chloride hydrate ($CaCl_2.2H_2O$: 147.01).

Method of preparation

Sodium Chloride	8.6 g
Potassium Chloride	0.3 g
Calcium Chloride Hydrate	0.33 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Ringer's Solution is a clear and colorless liquid. It has a slightly saline taste.

Identification (1) Evaporate 10 mL of Ringer's Solution to 5 mL: the solution responds to the Qualitative Tests <*1.09*> for potassium salt and calcium salt.

(2) Ringer's Solution responds to the Qualitative Tests <*1.09*> for sodium salt and chloride.

pH <*2.54*> 5.0 – 7.5

Purity (1) Heavy metals <*1.07*>—Evaporate 100 mL of Ringer's Solution to about 40 mL on a water bath. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Control solution: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic <*1.11*>—Perform the test with 20 mL of Ringer's Solution as the test solution (not more than 0.1 ppm).

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Chlorine—To 20 mL of Ringer's Solution, accurately measured, add 30 mL of water. Titrate <*2.50*> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of sodium fluorescein TS).

Each mL of 0.1 mol/L silver nitrate VS
$= 3.545$ mg of Cl

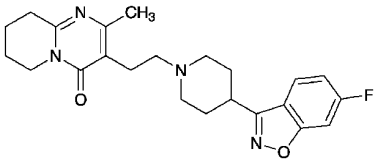
(2) Calcium chloride Hydrate—To 50 mL of Ringer's Solution, exactly measured, add 2 mL of 8 mol/L potassium hydroxide TS and 0.05 g of NN indicator, and titrate <*2.50*> immediately with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the color of the solution changes from red-purple to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
$= 1.470$ mg of $CaCl_2.2H_2O$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous infusions may be used.

Risperidone

リスペリドン



$C_{23}H_{27}FN_4O_2$: 410.48

3-{2-[4-(6-Fluoro-1,2-benzoisoxazol-3-yl)piperidin-1-yl]ethyl}-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one

[*106266-06-2*]

Risperidone contains not less than 98.5% and not more than 101.0% of $C_{23}H_{27}FN_4O_2$, calculated on the dried basis.

Description Risperidone occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Risperidone in 2-propanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Risperidone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 169 – 173°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Risperidone 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 0.10 g of Risperidone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than 1.5 times the peak area of risperidone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

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

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of ammonium acetate (1 in 200).

Mobile phase B: Methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	70	30
2 – 17	70 → 30	30 → 70
17 – 22	30	70

Flow rate: 1.5 mL per minute.

Time span of measurement: About 1.6 times as long as the retention time of risperidone.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7 to 13% 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 risperidone are not less than 10,000 and not more than 2.0, respectively.

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

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.16 g of Risperidone, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.52 mg of $C_{23}H_{27}FN_4O_2$

Containers and storage Containers—Tight containers.

Risperidone Fine Granules

リスペリドン細粒

Risperidone Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$: 410.48).

Method of preparation Prepare as directed under Granules, with Risperidone.

Identification To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

Purity Related substances—To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7.5 to 12.5% 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 risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Risperidone Fine Granules, equivalent to about 3 mg of risperidone ($C_{23}H_{27}FN_4O_2$) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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 5 mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying <*2.41*> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 15 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of risperidone of both solutions.

Dissolution rate (%) with respect to the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 54/5$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis
M_T: Amount (g) of sample
C: Labeled amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$) in 1 g

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 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 risperidone are not less than 3500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0%.

Particle size <*6.03*> It meets the requirements of Fine granules.

Assay If necessary powder Risperidone Fine Granules, and weigh accurately an amount, equivalent to about 2 mg of risperidone ($C_{23}H_{27}FN_4O_2$), add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately

about 50 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (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 sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of risperidone from each solution.

$$\text{Amount (mg) of risperidone } (C_{23}H_{27}FN_4O_2) = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

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

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 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 the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

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

Containers and storage Containers—Tight containers.

Risperidone Oral Solution

リスペリドン内服液

Risperidone Oral Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$: 410.48).

Method of preparation Prepare as directed under Liquids and Solutions for Oral Administration, with Risperidone.

Description Risperidone Oral Solution occurs as a clear and colorless liquid.

Identification To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add 50 mg of sodium hydrogen carbonate and 10 mL of diethyl ether, shake, centrifuge, and evaporate the supernatant liquid to dryness in lukewarm water. Determine the absorption spectrum of a solution of the residue in 100 mL of 2-propanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

pH Being specified separately.

Purity Related substances—To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add methanol to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of methanol and water (9:1) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7.5 to 12.5% 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 risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^1 CFU/mL, respectively. *Escherichia coli* is not observed.

Uniformity of dosage units <6.02> The granules in single-unit containers meet the requirement of the Mass variation test.

Assay To an exact volume of Risperidone Oral Solution, equivalent to about 2 mg of risperidone ($C_{23}H_{27}FN_4O_2$) according to the labeled amount, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add

10 mL of water, then add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of risperidone from each solution.

$$\text{Amount (mg) of risperidone } (C_{23}H_{27}FN_4O_2) = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

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

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 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 the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

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

Containers and storage Containers—Tight containers.

Risperidone Tablets

リスペリドン錠

Risperidone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$: 410.48).

Method of preparation Prepare as directed under Tablets, with Risperidone.

Identification Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

Purity Related substances—Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of risperidone, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with 10 μL of this solution is equivalent to 7.5 to 12.5% 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 risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Risperidone Tablets add $3V/5$ mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly V mL so that each mL contains 0.1 mg of risperidone ($C_{23}H_{27}FN_4O_2$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of risperidone } (C_{23}H_{27}FN_4O_2) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Tablets is not less than 75%.

Start the test with 1 tablet of Risperidone Tablets, with-

draw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly V' mL so that each mL contains about 0.56 μg of risperidone ($C_{23}H_{27}FN_4O_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of risperidone of both solutions.

Dissolution rate (%) with respect to the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/5$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis
C: Labeled amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$) in 1 tablet

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 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 risperidone are not less than 3500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Risperidone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of risperidone ($C_{23}H_{27}FN_4O_2$), add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (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 sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of risperidone from each solution.

Amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$)
$= M_S \times A_T/A_S \times 1/25$

M_S: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

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

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

System suitability—

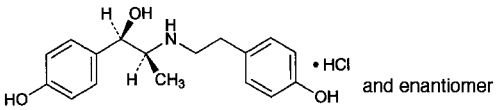
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

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

Containers and storage Containers—Tight containers.

Ritodrine Hydrochloride

リトドリン塩酸塩



$C_{17}H_{21}NO_3 \cdot HCl$: 323.81
(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-{[2-(4-hydroxyphenyl)ethyl]amino}propan-1-ol monohydrochloride
[*23239-51-2*]

Ritodrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{17}H_{21}NO_3.HCl$.

Description Ritodrine Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water, in methanol and in ethanol

(99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Ritodrine Hydrochloride (1 in 10) shows no optical rotation.

It is gradually colored to a light yellow by light.

Melting point: about 196°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ritodrine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ritodrine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ritodrine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ritodrine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ritodrine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 50 mL of water is between 4.5 and 5.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Ritodrine 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) Related substances—Dissolve 20 mg of Ritodrine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of ritodrine threo-isomer, having the relative retention time of about 1.2 with respect to ritodrine, obtained from the sample solution is not larger than 4/5 times the peak area of ritodrine from the standard solution, the area of the peak other than ritodrine and ritodrine threo-isomer is not larger than 3/10 times the peak area of ritodrine from the standard solution, and the total area of the peaks other than ritodrine and ritodrine threo-isomer is not larger than 4 times the peak area of ritodrine from the standard solution.

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

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

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

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add exactly 50 mL of the mobile phase. Confirm that the peak area of ritodrine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To 20 mg of Ritodrine Hydrochloride add 50 mL of the mobile phase and 5.6 mL of sulfuric acid, and add the mobile phase to make 100 mL. Heat a portion of this solution at about 85°C for about 2 hours, and allow to cool. Pipet 10 mL of this solution, and add exactly 10 mL of 2 mol/L sodium hydroxide TS. When the procedure is run with 10 μL of this solution under the above operating conditions, ritodrine and the threo-isomer 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Ritodrine Hydrochloride and Ritodrine Hydrochloride RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 25 mL of these solutions, add exactly 5 mL of the internal standard solution, then add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ritodrine to that of the internal standard.

$$\text{Amount (mg) of } C_{17}H_{21}NO_3.HCl = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of ritodrine 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, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less then 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ritodrine Hydrochloride Tablets

リトドリン塩酸塩錠

Ritodrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl: 323.81).

Method of preparation Prepare as directed under Tablets, with Ritodrine Hydrochloride.

Identification To 10 mL of the filtrate obtained in the Assay add 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 272 nm and 276 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ritodrine Hydrochloride Tablets add 9 mL of 0.01 mol/L hydrochloric acid TS, shake until the tablet is completely disintegrated, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL, and filter through a membrane filter having pore size of 0.45 μm. Pipet 3 mL of the filtrate, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 3 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 10,000).
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 under the above operating conditions, ritodrine 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Ritodrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ritodrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, 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 exactly 80 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ritodrine.

Dissolution rate (%) with respect to the labeled amount of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Ritodrine Hydrochloride RS
C: Labeled amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl) 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 80 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ritodrine are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 80 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 1.5%.

Assay To 20 Ritodrine Hydrochloride Tablets add 150 mL of 0.01 mol/L hydrochloric acid TS, shake for 20 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Filter through a glass filter (G4), and discard the first 20 mL of the filtrate. Pipet 30 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 30 mL of this solution, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> ac-

cording to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl) = $M_S \times Q_T/Q_S \times 4$

M_S: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of ritodrine 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, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

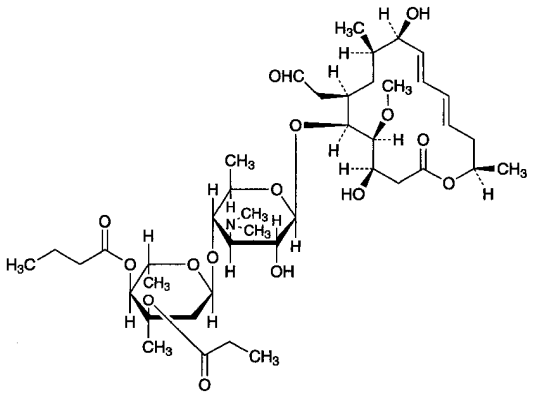
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Rokitamycin

ロキタマイシン



$C_{42}H_{69}NO_{15}$: 827.99

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Butanoyl-2,6-dideoxy-3-*C*-methyl-3-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

[*74014-51-0*]

Rokitamycin is a derivative of leucomycin A_5, which is a macrolide antibiotic produced by the growth of the mutants of *Streptomyces kitasatoensis*.

It contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Rokitamycin is expressed as mass (potency) of rokitamycin ($C_{42}H_{69}NO_{15}$).

Description Rokitamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in chloroform, freely soluble in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Rokitamycin in methanol (1 in 50,000), as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rokitamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rokitamycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Rokitamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Rokitamycin in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 20), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits single signals A, B, C and D at around δ 1.4 ppm, at around δ 2.5 ppm, at around δ 3.5 ppm and at around δ 9.8 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C:D, is

about 3:6:3:1.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Rokitamycin 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 Rokitamycin in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 3″-*O*-propionylleucomycin A_7 having the relative retention time of about 0.72, 3″-*O*-propionylisoleucomycin A_5 having the relative retention time of about 0.86 and 3″-*O*-propionylleucomycin A_1 having the relative retention time of about 1.36 with respect to rokitamycin obtained with the sample solution are not larger than the peak area of rokitamycin with the standard solution, the area of the peak other than rokitamycin, 3″-*O*-propionylleucomycin A_7, 3″-*O*-propionylisoleucomycin A_5 and 3″-*O*-propionylleucomycin A_1 is not larger than 23/100 times the peak area of rokitamycin with the standard solution, and the total area of the peaks other than rokitamycin is not larger than 3 times the peak area of rokitamycin with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside 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 55°C.

Mobile phase: A mixture of methanol, diluted 0.5 mol/L ammonium acetate TS (2 in 5) and acetonitrile (124:63:13).

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

Time span of measurement: About 2.5 times as long as the retention time of rokitamycin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add acetonitrile to make exactly 10 mL. Confirm that the peak area of rokitamycin obtained with 5 μL of this solution is equivalent to 7 to 13% of that with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rokitamycin are not less than 3000 and not more than 1.5, respectively.

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 rokitamycin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH ⟨*2.54*⟩ of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Rokitamycin RS equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Rokitamycin equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rokitamycin Tablets

ロキタマイシン錠

Rokitamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$: 827.99).

Method of preparation Prepare as directed under Tablets, with Rokitamycin.

Identification Take an amount of powdered Rokitamycin Tablets, equivalent to 10 mg (potency) of Rokitamycin according to the labeled amount, add 20 mL of methanol, and centrifuge if necessary. To 1 mL of this solution add methanol to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 230 nm and 233 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 50 mL of water to 1 tablet of Rokitamycin Tablets, and disintegrate. Then add 10 mL of methanol, shake well, and add water to make exactly 100 mL. Centrifuge this solution if necessary, filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μg (potency) of Rokitamycin, and use this solution as the

sample solution. Separately, weigh accurately about 20 mg (potency) of Rokitamycin RS, dissolve in 10 mL of methanol, 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 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$)
$= M_S \times A_T/A_S \times V'/V \times 1/10$

M_S: Amount [mg (potency)] of Rokitamycin RS

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Rokitamycin Tablets is not less than 80%.

Start the test with 1 tablet of Rokitamycin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first 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 Rokitamycin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Rokitamycin RS, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of the 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 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Rokitamycin RS
C: Labeled amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$) in 1 tablet

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> under the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Rokitamycin.

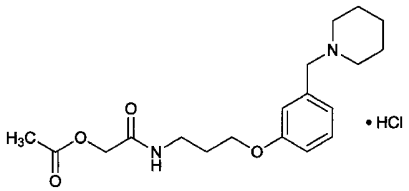
(ii) Sample solutions—Weigh accurately not less than 20 tablets of Rokitamycin Tablets, and powder. Weigh accurately an amount of contents, equivalent to about 40 mg (potency) of Rokitamycin, add 50 mL of methanol, shake vigorously, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and centrifuge if necessary. Measure exactly a suitable quantity of this solution, add polysorbate 80 solution, prepared by adding 0.1 mol/L phosphate buffer solution, pH 8.0 to 0.1 g of polysorbate 80 to make 1000 mL, so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Roxatidine Acetate Hydrochloride

ロキサチジン酢酸エステル塩酸塩



$C_{19}H_{28}N_2O_4 \cdot HCl$: 384.90
(3-{3-[(Piperidin-1-yl)methyl]phenoxy}propylcarbamoyl)methyl acetate monohydrochloride
[*93793-83-0*]

Roxatidine Acetate Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{28}N_2O_4$.HCl.

Description Roxatidine Acetate Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Roxatidine Acetate Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Roxatidine Acetate Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Roxatidine Acetate Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Roxatidine Acetate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Roxatidine Acetate Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 6.0.

Melting point <*2.60*> 147 – 151°C (after drying).

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

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Roxatidine Acetate 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) Related substances—Dissolve 50 mg of Roxatidine Acetate Hydrochloride in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>

according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than roxatidine acetate obtained from sample solution is not larger than 1/5 times the peak area of roxatidine acetate from the standard solution, and the total area of the peaks other than roxatidine acetate is not larger than 1/2 times the peak area of roxatidine acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

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

Time span of measurement: About 1.5 times as long as the retention time of roxatidine acetate beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of roxatidine acetate obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 50 mg of roxatidine acetate hydrochloride and 10 mg of benzoic acid in 25 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above operating conditions, benzoic acid and roxatidine acetate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Roxatidine Acetate Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.49 mg of $C_{19}H_{28}N_2O_4$.HCl

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Capsules

ロキサチジン酢酸エステル塩酸塩徐放カプセル

Roxatidine Acetate Hydrochloride Extended-release Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Capsules, with Roxatidine Acetate Hydrochloride.

Identification To 1 mL of the filtrate obtained in the Assay add ethanol (99.5) to make 20 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 275 nm and 278 nm, and between 282 nm and 285 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, add exactly V mL of ethanol (99.5) so that each mL contains about 2.5 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl), disperse the particles with the aid of ultrasonic wave, and filter through a membrane filter with pore size of not more than 1.0 μm. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) = $M_S \times Q_T/Q_S \times V/20$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rates of a 37.5-mg capsule in 45 minutes, in 90 minutes and in 8 hours are 10 – 40%, 35 – 65%, and not less than 70%, respectively, and of a 75-mg capsule in 60 minutes, in 90 minutes and in 8 hours are 20 – 50%, 35 – 65%, and not less than 70%, respectively.

Start the test with 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, withdraw exactly 20 mL of the medium at the specified minute after starting the test, and supply exactly 20 mL of warmed water to 37 ± 0.5°C immediately after withdrawing of the medium every time, and filter the media withdrawn 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 42 μg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 50

mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solutions and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, $A_{T(n)}$ and A_S, of roxatidine acetate of each solution.

Dissolution rate (%) with respect to the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) on the nth medium withdrawing (n = 1,2,3)

$$= M_S \times \left[\frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1}\left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45}\right)\right] \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

C: Labeled amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 5 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 roxatidine acetate are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Assay Take out the contents of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl), add exactly 30 mL of ethanol (99.5), shake, and filter through a membrane filter with pore size of not more than 1.0 μm. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in ethanol (99.5) to make exactly 20 mL. To exactly 8 mL of this solution add exactly 2 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl)
$= M_S \times Q_T/Q_S \times 3/2$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate 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 roxatidine acetate are elute 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Tablets

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Roxatidine Acetate Hydrochloride Extended-release Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Tablets, with Roxatidine Acetate Hydrochloride.

Identification Powder Roxatidine Acetate Hydrochloride Extended-release Tablets. To a portion of the powder, equivalent to 37.5 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 40 mL of ethanol (99.5), and disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking. After shaking thoroughly, add ethanol (99.5) to make 50 mL. Filter the solution, and to 4 mL of the filtrate add ethanol (99.5) to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

Uniformity of dosage units <*6.02*> Perform the test accord-

ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Roxatidine Acetate Hydrochloride Extended-release Tablets add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves with occasional shaking, then add 7.5 mL of acetonitrile, then agitate again for 5 minutes with the aid of ultrasonic waves. Add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves, shake thoroughly, add a mixture of water, triethylamine and acetic acid (100) (340:2:1) to make exactly 50 mL, centrifuge, and filter the supernatant liquid. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to 6 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl), add exactly 3 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) $= M_S \times Q_T/Q_S \times 8/V$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 37.5 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl), add 40 mL of the mobile phase, and agitate for 10 minutes with the aid of ultrasonic waves with occasional shaking. Further shake thoroughly, add the mobile phase to make exactly 50 mL, centrifuge, and filter the supernatant liquid. Discard the first 10 mL of the filtrate, pipet 8 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 38 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 8 mL of this solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 20 mL, 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 <*2.01*> under the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 mm in inside 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 water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine 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, the internal standard and roxatidine acetate 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Roxatidine Acetate Hydrochloride for Injection

注射用ロキサチジン酢酸エステル塩酸塩

Roxatidine Acetate Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Injections, with Roxatidine Acetate Hydrochloride.

Description It occurs as white masses or powder.

Identification To an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 30 mL of ethanol (99.5), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate add ethanol (99.5) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 275 nm and 279 nm and between 282 nm and 286 nm.

pH Being specified separately.

Purity Clarity and color of solution Dissolve an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, in 20 mL of isotonic sodium chloride solution: the solution is clear and colorless.

Bacterial endotoxins <*4.01*> Less than 4.0 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirements of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve with water each content of 10 Roxatidine Acetate Hydrochloride for Injection, wash the containers with water, combine the solution of the content and washings, and add water to make exactly V mL so that each mL contains about 3.75 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl). Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 4 hours, dissolve in water to make exactly 50 mL. Pipet 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) in 1 Roxatidine Acetate Hydrochloride for Injection
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—Dissolve 20 mg of guanine in 10 mL of 2 mol/L hydrochloric acid TS, add 50 mL of water, then add 20 mL of a solution of sodium hydroxide (1 in 25) and water to make 100 mL. To 10 mL of this solution add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 14 minutes.

System suitability—

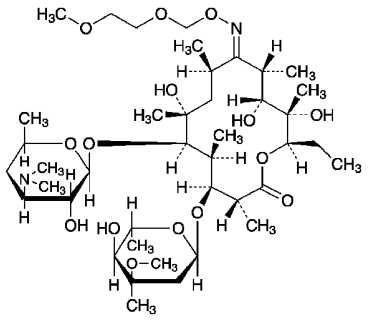
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Roxithromycin

ロキシスロマイシン



$C_{41}H_{76}N_2O_{15}$: 837.05
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,9*E*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-9-(2-methoxyethoxy)methoxyimino-2,4,6,8,10,12-hexamethylpentadecan-13-olide
[*80214-83-1*]

Roxithromycin is a derivative of erythromycin.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Roxithromycin is expressed as mass (potency) of roxithromycin ($C_{41}H_{76}N_2O_{15}$).

Description Roxithromycin occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, sparingly soluble in acetonitrile, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Roxithromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Roxithromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -93 – $-96°$ (0.5 g calculated on the anhydrous basis, acetone, 50 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Roxithromycin 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 exactly 40 mg of Roxithromycin in the mobile phase A to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 20 mg of Roxithromycin RS in the mobile phase A to make exactly 10 mL. 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 exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak

areas by the automatic integration method: the area of a peak having the relative retention time of about 1.05 with respect to roxithromycin from the sample solution is not larger than 2 times of the peak area of roxithromycin from the standard solution. The area of the peak other than the peak of roxithromycin and the peak having the relative retention time of about 1.05 is not larger than the peak area of roxithromycin from the standard solution, and the total area of the peaks other than roxithromycin from the sample solution is not larger than 6 times of the peak area of roxithromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside 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: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Mobile phase B: A mixture of acetonitrole and water (7:3).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 38	100	0
38 – 39	100 → 90	0 → 10
39 – 80	90	10

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 21 minutes.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 10 mL. Confirm that the peak area of roxithromycin obtained from 20 μL of this solution is equivalent to 15 to 25% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg each of Roxithromycin RS and *N*-demethylroxithromycin in the mobile phase A to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Roxithromycin and Roxithromycin RS, equivalent to about 20 mg (potency), and dissolve separately in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of roxithromycin.

$$\text{Amount [}\mu\text{g (potency)] of } C_{41}H_{76}N_2O_{15} = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Roxithromycin RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside 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: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

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

System suitability—

System performance: Dissolve 5 mg each of Roxithromycin RS and *N*-demethylroxithromycin in the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6 and the symmetry factor of the peak of roxithromycin is not more than 1.5.

System repeatability: When, the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Freeze-dried Live Attenuated Rubella Vaccine

乾燥弱毒生風しんワクチン

Freeze-dried Live Attenuated Rubella Vaccine is a preparation for injection which is dissolved before use.

It contains live attenuated rubella virus.

It conforms to the requirements of Freeze-dried Live Attenuated Rubella Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Rubella Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Saccharated Pepsin

含糖ペプシン

Saccharated Pepsin is a mixture of pepsin obtained from the gastric mucosa of hog or cattle and Lactose Hydrate, and it is an enzyme drug having a proteolytic activity.

It contains not less than 3800 units and not more than 6000 units per g.

Description Saccharated Pepsin occurs as a white powder. It has a characteristic odor, and has a slightly sweet taste.

It dissolves in water to give a slightly turbid liquid, and does not dissolve in ethanol (95) and in diethyl ether.

It is slightly hygroscopic.

Purity **(1)** Rancidity—Saccharated Pepsin has no unpleasant or rancid odor.

(2) Acidity—Dissolve 0.5 g of Saccharated Pepsin in 50 mL of water, and add 0.50 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution is red in color.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay (i) Substrate solution—Use the substrate solution 1 described in (2) Assay for protein digestive activity under the Digestion Test ⟨*4.03*⟩ after adjusting the pH to 2.0.

(ii) Sample solution—Weigh accurately an amount of Saccharated Pepsin equivalent to about 1250 units, dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make exactly 50 ml.

(iii) Standard solution—Weigh accurately a suitable amount of Saccharated Pepsin RS, and dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make a solution containing about 25 units per mL.

(iv) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test ⟨*4.03*⟩, and determine the absorbances, A_T and A_{TB}, of the sample solution, using trichloroacetic acid TS A as the precipitation reagent. Separately, determine the absorbances, A_S and A_{SB}, of the standard solution in the same manner as the sample solution.

$$\text{Units in 1 g of Saccharated Pepsin} = U_S \times (A_T - A_{TB})/(A_S - A_{SB}) \times 1/M$$

U_S: Units per mL of the standard solution

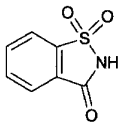
M: Amount (g) of Saccharated Pepsin per mL of the sample solution

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Saccharin

サッカリン



$C_7H_5NO_3S$: 183.18

1,2-Benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide

[*81-07-2*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Saccharin contains not less than 99.0% and not more than 101.0% of $C_7H_5NO_3S$, calculated on the dried basis.

◆**Description** Saccharin occurs as colorless or white crystals or a white crystalline powder. It has a very sweet taste.

It is sparingly soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.◆

◆**Identification** Determine the infrared absorption spectrum of Saccharin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Melting point ⟨*2.60*⟩ 226 – 230°C

Purity ◆**(1)** Clarity and color of solution—Dissolve 1.0 g of Saccharin in 30 mL of hot water or in 50 mL of ethanol (95): the solution is clear and colorless in each case.◆

◆**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Saccharin 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) Benzoate and salicylate—To 10 mL of a saturated solution of Saccharin in hot water add 3 drops of iron (III) chloride TS: no precipitate is formed, and no red-purple to purple color develops.

◆**(4)** *o*-Toluene sulfonamide—Dissolve 10 g of Saccharin in 70 mL of sodium hydroxide TS, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, then evaporate the solvent. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of *o*-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate to dryness on a water bath, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of *o*-toluene sulfonamide to that of the internal standard: Q_T is

not more than Q_S.

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated 3% with diethylene glycol succinate polyester for gas chromatography (180 – 250 μm in particle diameter).

Column temperature: A constant temperature of about 200°C.

Temperature of injection port: A constant temperature of about 225°C.

Temperature of detector: A constant temperature of about 250°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.◆

(5) Readily carbonizable substances <*1.15*>—Perform the test with 0.20 g of Saccharin, by warming at 48 to 50°C for 10 minutes: the color of the solution is not more intense than the matching fluid A.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Saccharin, dissolve in 40 mL of ethanol (95), add 40 mL of water, and titrate <*2.50*> 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.

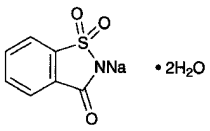
Each mL of 0.1 mol/L sodium hydroxide VS
= 18.32 mg of $C_7H_5NO_3S$

Containers and storage Containers—Well-closed containers.

Saccharin Sodium Hydrate

Saccharin Sodium

サッカリンナトリウム水和物



$C_7H_4NNaO_3S.2H_2O$: 241.20

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide dihydrate

[*6155-57-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Saccharin Sodium Hydrate contains not less than 99.0% and not more than 101.0% of saccharin sodium ($C_6H_4NNaO_3S$: 205.17), calculated on the anhydrous basis.

◆**Description** Saccharin Sodium Hydrate occurs as colorless crystals or a white, crystalline powder. It has an intensely sweet taste, even in 10,000 dilutions.

It is freely soluble in water and in methanol, and sparingly soluble in ethanol (95) and in acetic acid (100).

It effloresces slowly and loses about half the amount of water of crystallization in air.◆

Identification ◆**(1)** Determine the infrared absorption spectrum of Saccharin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

(2) A solution of Saccharin Sodium Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity ◆**(1)** Clarity and color of solution—Dissolve 1.0 g of Saccharin Sodium Hydrate in 1.5 mL of water or in 50 mL of ethanol (95): the solution is clear and colorless.◆

(2) Acidity or alkalinity—Dissolve 1.0 g of Saccharin Sodium Hydrate in 10 mL of water, and add 1 drop of phenolphthalein TS: the solution is colorless. Add 1 drop of 0.1 mol/L sodium hydroxide VS to the solution: the color changes to red.

◆**(3)** Heavy metals <*1.07*>—Dissolve 2.0 g of Saccharin Sodium Hydrate in 40 mL of water, add 0.7 mL of dilute hydrochloric acid, dilute with water to make 50 mL, and rub the inner wall of the vessel with a glass rod until crystallization begins. Allow the solution to stand for 1 hour after the beginning of crystallization, and then filter through dry filter paper. Reject the first 10 mL of the filtrate, and take 25 mL of the subsequent filtrate. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test, using this solution as the test solution. To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 10 ppm).◆

(4) Benzoate and salicylate—Dissolve 0.5 g of Saccharin

Sodium Hydrate in 10 mL of water, add 5 drops of acetic acid (31) and 3 drops of iron (III) chloride TS: no turbidity is produced, and no red-purple to purple color develops.

◆**(5)** *o*-Toluene sulfonamide—Dissolve 10 g of Saccharin Sodium Hydrate in 50 mL of water, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, and evaporate ethyl acetate. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of *o*-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate on a water bath to dryness, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of *o*-toluene sulfonamide to that of the internal standard: Q_T is not more than Q_S.

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography (180 to 250 μm in diameter), coated with diethyleneglycol succinate polyester for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 200°C.

Injection port temperature: A constant temperature of about 225°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.◆

(6) Readily carbonizable substances <*1.15*>—Perform the test with 0.20 g of Saccharin Sodium Hydrate. Allow the solution to stand between 48°C and 50°C for 10 minutes: the solution has no more color than Matching Fluid A.

Water <*2.48*> Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.15 g of Saccharin Sodium Hydrate, dissolve in 50 mL of acetic acid (100), heat slightly if necessary, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

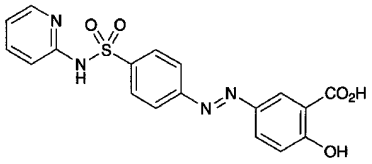
Each mL of 0.1 mol/L perchloric acid VS
= 20.52 mg of $C_7H_4NNaO_3S$

◆**Containers and storage** Containers—Well-closed containers.◆

Salazosulfapyridine

Sulfasalazine

サラゾスルファピリジン



$C_{18}H_{14}N_4O_5S$: 398.39

2-Hydroxy-5-[4-(pyridin-2-ylsulfamoyl)phenylazo]benzoic acid

[*599-79-1*]

Salazosulfapyridine, when dried, contains not less than 96.0% of $C_{18}H_{14}N_4O_5S$.

Description Salazosulfapyridine occurs as a yellow to yellow-brown, fine powder. It is odorless and tasteless.

It is sparingly soluble in pyridine, slightly soluble in ethanol (95), practically insoluble in water, in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: 240 – 249°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Salazosulfapyridine in 20 mL of dilute sodium hydroxide TS: a red-brown color develops. This color gradually fades upon gradual addition of 0.5 g of sodium hydrosulfite with shaking. Use this solution in the following tests (2) to (4).

(2) To 1 mL of the solution obtained in (1) add 40 mL of water, neutralize with 0.1 mol/L hydrochloric acid TS, and add water to make 50 mL. To 5 mL of this solution add 2 to 3 drops of dilute iron (III) chloride TS: a red color develops and changes to purple, then fades when dilute hydrochloric acid is added dropwise.

(3) The solution obtained in (1) responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(4) To 1 mL of the solution obtained in (1) add 1 mL of pyridine and 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(5) Determine the absorption spectrum of a solution of Salazosulfapyridine in dilute sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Chloride <*1.03*>—Dissolve 2.0 g of Salazosulfapyridine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of nitric acid, shake, 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 ⟨*1.14*⟩—Dissolve 2.0 g of Salazosulfapyridine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of hydrochloric acid, shake, 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 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Salazosulfapyridine 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 ⟨*1.11*⟩—Take 1.0 g of Salazosulfapyridine in a decomposition flask, add 20 mL of nitric acid, and heat gently until it becomes fluid. After cooling, add 5 mL of sulfuric acid, and heat until white fumes are evolved. Add, if necessary, 5 mL of nitric acid after cooling, and heat again. Repeat this operation until the solution becomes colorless to slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again. After cooling, add water to make 25 mL. Perform the test with 5 mL of this solution as the test solution: the color of the test solution is not deeper than that of the following color standard.

Color standard: Proceed in the same manner without Salazosulfapyridine, transfer 5 mL of the obtained solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Salazosulfapyridine in 20 mL of pyridine, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add pyridine 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with diluted methanol (9 in 10) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Salicylic acid—To 0.10 g of Salazosulfapyridine add 15 mL of diethyl ether, and shake vigorously. Add 5 mL of dilute hydrochloric acid, shake vigorously for 3 minutes, collect the diethyl ether layer, and filter. To the water layer add 15 mL of diethyl ether, shake vigorously for 3 minutes, collect the diethyl ether layer, filter, and combine the filtrates. Wash the residue on the filter paper with a small quantity of diethyl ether, and combine the washings and the filtrate. Evaporate the diethyl ether with the aid of air-stream at room temperature. To the residue add dilute ammonium iron (III) sulfate TS, shake, and filter, if necessary. Wash the residue on the filter paper with a small quantity of dilute ammonium iron (III) sulfate TS, combine the washings and the filtrate, add dilute ammonium iron (III) sulfate TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, dissolve in dilute ammonium iron (III) sulfate TS to make exactly 400 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 535 nm of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: salicylic acid content is not more than 0.5%.

$$\text{Content (\%) of salicylic acid } (C_7H_6O_3) = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of salicylic acid for assay

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

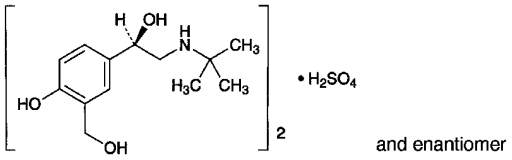
Assay Weigh accurately about 20 mg of Salazosulfapyridine, previously dried, and perform the test as directed in the procedure of determination for sulfur under the Oxygen Flask Combustion Method ⟨*1.06*⟩, using 10 mL of diluted hydrogen peroxide (30) (1 in 40) as an absorbing liquid.

$$\text{Each mL of 0.005 mol/L barium perchlorate VS} = 1.992 \text{ mg of } C_{18}H_{14}N_4O_5S$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Salbutamol Sulfate

サルブタモール硫酸塩



$(C_{13}H_{21}NO_3)_2.H_2SO_4$: 576.70
(1*RS*)-2-(1,1-Dimethylethyl)amino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol hemisulfate
[*51022-70-9*]

Salbutamol Sulfate, when dried, contains not less than 98.0% of $(C_{13}H_{21}NO_3)_2.H_2SO_4$.

Description Salbutamol Sulfate occurs as a white powder.

It is freely soluble in water, slightly soluble in ethanol (95), and in acetic acid (100) and practically insoluble in diethyl ether.

A solution of Salbutamol Sulfate (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Salbutamol Sulfate in 0.1 mol/L hydrochloric acid TS (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Salbutamol Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Salbutamol Sulfate (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g

of Salbutamol Sulfate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Salbutamol 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).

(3) Related substances—Dissolve 20 mg of Salbutamol Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water and ammonia solution (28) (25:15:8:2) to a distance of about 15 cm, and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

(4) Boron—Take 50 mg of Salbutamol Sulfate and 5.0 mL of the Standard Boron Solution, and transfer to a platinum crucible. Add 5 mL of potassium carbonate-sodium carbonate TS, evaporate on a water bath to dryness, and dry at 120°C for 1 hour. Ignite the residue immediately. After cooling, add 0.5 mL of water and 3 mL of curcumin TS to the residue, warm gently in a water bath for 5 minutes. After cooling, add 3 mL of acetic acid-sulfuric acid TS, mix, and allow to stand for 30 minutes. Add ethanol (95) to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution and standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using ethanol (95) as the blank: the absorbance of the sample solution at 555 nm is not larger than that of the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

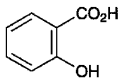
Assay Weigh accurately about 0.9 g of Salbutamol Sulfate, previously dried, and dissolve in 50 mL of acetic acid (100) by warming. After cooling, titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 57.67 mg of $(C_{13}H_{21}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.

Salicylic Acid

サリチル酸



$C_7H_6O_3$: 138.12
2-Hydroxybenzoic acid
[*69-72-7*]

Salicylic Acid, when dried, contains not less than 99.5% and not more than 101.0% of $C_7H_6O_3$.

Description Salicylic Acid occurs as white crystals or crystalline powder. It has a slightly acid, followed by an acrid taste.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.

Identification **(1)** A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests ⟨*1.09*⟩ (1) and (3) for salicylate.

(2) Determine the absorption spectrum of a solution of Salicylic Acid in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Salicylic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 158 – 161°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to 100 mL, and filter. Discard the first 20 mL of the filtrate, take subsequent 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 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.008%).

(2) Sulfate ⟨*1.14*⟩—To 20 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 ⟨*1.07*⟩—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide TS, 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 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Related substances—Dissolve 0.50 g of Salicylic Acid in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve exactly 10 mg of phenol, exactly 25 mg of 4-hydroxyisophthalic acid and exactly 50 mg of parahydroxybenzoic acid 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol obtained from the sample solution are not larger than the area of each respective peak from the standard solution, the area of the peak other than salicylic acid and other than the substances mentioned above is not larger than the peak area of 4-hydroxisophthalic acid from the standard solution, and the total area of peaks other than salicylic acid is not larger than 2 times the peak area of parahydroxybenzoic acid from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (60:40:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of salicylic acid, beginning after the solvent peak.

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 areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol obtained from 10 μL of this solution are equivalent to 14 to 26% of the area of each respective peak from 10 μL of the standard solution.

System performance: Dissolve 10 mg of phenol, 25 mg of 4-hydroxyisophthalic acid and 50 mg of parahydroxybenzoic acid in 100 mL of the mobile phase. 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, parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol are eluted in this order with the resolution between the peaks of 4-hydroxyisophthalic acid and phenol being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol is not more than 2.0%, respectively.

Loss on drying <2.41> Not more than 0.5% (2 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Salicylic Acid, previously dried, dissolve in 25 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 13.81 mg of $C_7H_6O_3$

Containers and storage Containers—Well-closed containers.

Salicylated Alum Powder

サリチル·ミョウバン散

Salicylated Alum Powder contains not less than 2.7% and not more than 3.3% of salicylic acid ($C_7H_6O_3$: 138.12).

Method of preparation

Salicylic Acid, finely powdered	30 g
Dried Aluminum Potassium Sulfate, very finely powdered	640 g
Talc, very finely powdered	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Salicylated Alum Powder occurs as a white powder.

Identification **(1)** The colored solution obtained in the Assay has a red-purple color and exhibits an absorption maximum <2.24> between 520 nm and 535 nm (salicylic acid).

(2) Shake 0.3 g of Salicylated Alum Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of salicylic acid in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R*f value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Assay Weigh accurately about 0.33 g of Salicylated Alum Powder, add 80 mL of ethanol (95), and shake vigorously. Dilute with ethanol (95) to make exactly 100 mL, filter, and discard the first 10 mL of the filtrate. Use the subsequent filtrate as the sample solution. Dissolve about 0.1 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in sufficient ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution into stoppered test tubes respectively, to each add exactly 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200), and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 25 mL. Determine the absorbances, A_T and A_S, of both solutions at 530 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with ethanol (95), instead of the sample

solution, as the blank.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_S \times A_T/A_S \times 1/10$$

M_S: Amount (mg) of salicylic acid for assay

Containers and storage Containers—Well-closed containers.

Salicylic Acid Adhesive Plaster

サリチル酸絆創膏

Method of preparation

Adhesive Plaster consists of a mixture of the below ingredients with carefully selected rubber, resins, zinc oxide and other substances. It has adhesive properties. It spreads evenly on a fabric.

Salicylic Acid, finely powdered	500 g
Adhesive plaster base	a sufficient quantity
To make	1000 g

Description The surface of Salicylic Acid Adhesive Plaster is whitish in color and adheres well to the skin.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Salicylic Acid Spirit

サリチル酸精

Salicylic Acid Spirit contains not less than 2.7 w/v% and not more than 3.3 w/v% of salicylic acid ($C_7H_6O_3$: 138.12).

Method of preparation

Salicylic Acid	30 g
Glycerin	50 mL
Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description Salicylic Acid Spirit is a clear, colorless liquid.

Specific gravity d_{20}^{20}: about 0.86

Identification The solution obtained in the Assay has a red-purple color. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 520 nm and 535 nm (salicylic acid).

Alcohol number <*1.01*> Not less than 8.8 (Method 2).

Assay Measure exactly 10 mL of Salicylic Acid Spirit, add 10 mL of ethanol (95) and water to make exactly 100 mL. Pipet 3 mL of this solution, and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL. Use this solution as the sample solution. Dissolve about 0.3 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in 10 mL of alcohol and water to make exactly 100 mL. Pipet 3 mL of this solution, dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add exactly 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200), dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to exactly 25 mL. Determine the absorbances <*2.24*>, A_T and A_S, of both solutions at 530 nm, using a blank solution prepared in the same manner with water instead of the sample solution.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of salicylic acid for assay

Containers and storage Containers—Tight containers.

Compound Salicylic Acid Spirit

複方サリチル酸精

Compound Salicylic Acid Spirit contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Salicylic Acid	20 g
Liquefied Phenol	5 mL
Glycerin	40 mL
Ethanol	800 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description Compound Salicylic Acid Spirit is a clear, colorless to light red liquid.

Specific gravity d_{20}^{20}: about 0.88

Identification **(1)** To 1 mL of Compound Salicylic Acid Spirit add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(2) To 1 mL of Compound Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 20 mL of diethyl ether. Wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, allow to stand for 10 minutes, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(3) To 0.5 mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution (1). To 2

mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chrloroform, wash the extract with two 5-mL portions of sodium hydrogen carbonate TS, and use the chloroform extract as the sample solution (2). Separately, dissolve 0.01 g each of salicylic acid and phenol in 5 mL each of chloroform, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solutions (1) and (2) 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, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution (1) and standard solution (1) show the same *R*f value, and the spots from the sample solution (2) and the standard solution (2) show the same *R*f value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution (1) and the corresponding spot from the sample solution (1) reveal a purple color.

Alcohol number <1.01> Not less than 7.5 (Method 2).

Assay Measure accurately 2 mL of Compound Salicylic Acid Spirit, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 50 mg of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/5$$

$$\text{Amount (mg) of phenol } (C_6H_6O) = M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/5$$

M_{Sa}: Amount (mg) of salicylic acid for assay
M_{Sb}: Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 1250).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography, 5 μm in particle diameter.

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

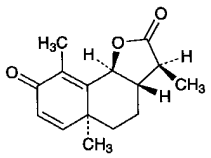
Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Santonin

サントニン



$C_{15}H_{18}O_3$: 246.30
(3*S*,3a*S*,5a*S*,9b*S*)-3,5a,9-Trimethyl-3a,5,5a,9b-tetrahydronaphtho[1,2-*b*]furan-2,8(3*H*,4*H*)-dione
[*481-06-1*]

Santonin, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{18}O_3$.

Description Santonin occurs as colorless crystals, or a white, crystalline powder.

It is freely soluble in chloroform, sparingly soluble in ethanol (95), and practically insoluble in water.

It becomes yellow by light.

Identification **(1)** Determine the absorption spectrum of a solution of Santonin in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Santonin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −170 – −175° (0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 172 – 175°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Santonin 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) Alkaloids—Boil 0.5 g of Santonin with 20 mL of diluted sulfuric acid (1 in 100), cool, and filter. Dilute 10 mL of the filtrate with water to 30 mL, add 3 drops of iodine TS, and allow to stand for 3 hours: no turbidity is produced.

(3) Artemisin—Dissolve 1.0 g of powdered Santonin in 2 mL of chloroform by slight warming: the solution is clear and colorless, or any yellow color produced is not darker than Matching Fluid A.

(4) Phenols—Boil 0.20 g of Santonin with 10 mL of water, cool, and filter. To the filtrate add bromine TS until

the color of the solution becomes yellow: no turbidity is produced.

(5) Acid-coloring substances—Moisten 10 mg of Santonin with nitric acid: no color develops immediately. Moisten Santonin with sulfuric acid, previously cooled to 0°C: no color is produced immediately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

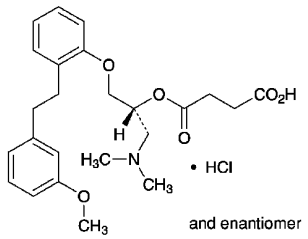
Assay Weigh accurately about 0.25 g of Santonin, previously dried, dissolve in 10 mL of ethanol (95) by warming, add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and heat on a water bath under a reflux condenser for 5 minutes. Cool quickly, and titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.63 mg of $C_{15}H_{18}O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sarpogrelate Hydrochloride

サルポグレラート塩酸塩



$C_{24}H_{31}NO_6$.HCl: 465.97
(2*RS*)-1-Dimethylamino-3-{2-[2-(3-methoxyphenyl)ethyl]phenoxy}propan-2-yl hydrogen succinate monohydrochloride
[*135159-51-2*]

Sarpogrelate Hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{24}H_{31}NO_6$.HCl, calculated on the anhydrous basis.

Description Sarpogrelate Hydrochloride occurs as a white crystalline powder.

It is slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Sarpogrelate Hydrochloride (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Sarpogrelate Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Sarpogrelate Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sarpogrelate Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Sarpogrelate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS separately with acetone by heating and suspending, filter and dry the crystals, and perform the test with the crystals.

(3) Dissolve 0.3 g of Sarpogrelate Hydrochloride in 6 mL of sodium hydroxide TS, shake well, allow to stand for 10 minutes, and filter. To 1 mL of the filtrate add 1 mL of dilute nitric acid. This solution responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Sarpogrelate 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Sarpogrelate Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Carry out the determination within 3 hours after preparing the sample solution. Dissolve 20 mg of Sarpogrelate Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, obtained from the sample solution is not larger than 1/5 times that of sarpogrelate from the standard solution, the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution, and the total area of the peaks other than sarpogrelate from the sample solution is not larger than 1/2 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 50 mg of Sarpogrelate Hydrochloride in 20 mL of water, and use as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution,

and add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ Not more than 0.5% (1 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Sarpogrelate Hydrochloride and Sarpogrelate Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Sarpogrelate Hydrochloride), add to them exactly 2.5 mL of the internal standard solution, and dissolve them with the mobile phase to make 50 mL. To 5 mL each of these solutions add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sarpogrelate to that of the internal standard.

$$\text{Amount (mg) of sarpogrelate hydrochloride } (C_{24}H_{31}NO_6.HCl) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside 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 water, acetonitrile and trifluoroacetic acid (1300:700:1).

Flow rate: Adjust the flow rate so that the retention time of sarpogrelate 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, sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sarpogrelate Hydrochloride Fine Granules

サルポグレラート塩酸塩細粒

Sarpogrelate Hydrochloride Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$: 465.97).

Method of preparation Prepare as directed under Granules, with Sarpogrelate Hydrochloride.

Identification To an amount of Sarpogrelate Hydrochloride Fine Granules, equivalent to 50 mg of Sarpogrelate Hydrochloride according to the labeled amount, add 10 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at room temperature for 10 minutes, then add 0.01 mol/L hydrochloric acid TS to make 100 mL, and disperse the particles with the aid of ultrasonic waves. Centrifuge this solution, and to 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 269 nm and 273 nm and between 274 nm and 278 nm.

Purity Related substances—Perform the procedure within 3 hours after preparing the sample solution. Powder Sarpogrelate Hydrochloride Fine Granules. To a portion of the powder, equivalent to 0.10 g of Sarpogrelate Hydrochloride according to the labeled amount, add 50 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. Filter thorough 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 sample solution. Pipet 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, obtained from the sample solution is not larger than 2.5 times that of sarpogrelate from the standard solution, and the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate, beginning after the solvent peak.

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 sarpogrelate obtained with 10

μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution, and add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Sarpogrelate Hydrochloride Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Sarpogrelate Hydrochloride Fine Granules add exactly $V/10$ mL of the internal standard solution, and add $4V/5$ mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Sarpogrelate Hydrochloride Fine Granules is not less than 85%.

Start the test with an accuretely weighed amount of Sarpogrelate Hydrochloride Fine Granules, equivalent to about 50 mg of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 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, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S/M_T \times A_T/A_S \times 1/C \times 180$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis
M_T: Amount (g) of sample
C: Labeled amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl) in 1 g

Particle size ⟨*6.03*⟩ It meets the requirements of Fine granules.

Assay Powder Sarpogrelate Hydrochloride Fine Granules. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), add exactly 25 mL of the internal standard solution, add about 200 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. To this solution add the mobile phase to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Sarpogrelate Hydroxide), add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sarpogrelate to that of the internal standard.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sarpogrelate Hydrochloride Tablets

サルポグレラート塩酸塩錠

Sarpogrelate Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl: 465.97).

Method of preparation Prepare as directed under Tablets, with Sarpogrelate Hydrochloride.

Identification Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 50 mg of Sarpogrelate Hydrochloride according to the labeled amount, add 10 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at room temperature for 10 minutes, then add 0.01 mol/L hydrochloric acid TS to make 100 mL, and disperse the particles with the aid of ultrasonic waves. Centrifuge this solution, and to 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 269 nm and 273 nm, and between 274 nm and 278 nm.

Purity Related substances—Perform the procedure within 12 hours after preparing the sample solution. Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 0.10 g of Sarpogrelate Hydrochloride according to the labeled amount, add 50 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. Filter the solution 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 sample solution. Pipet 2 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, obtained from the sample solution is not larger than 1.5 times that of sarpogrelate from the standard solution, and the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the stock solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution, and add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Sarpogrelate Hydrochloride Tablets add exactly V/10 mL of the internal standard solution, and disintegrate the tablet. Add 4V/5 mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sarpogrelate Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Sarpogrelate Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 55.6 μg of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water <2.48> in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 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, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis
C: Labeled amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Sarpogrelate Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), add exactly 25 mL of the internal standard solution, add about 200 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. To this solution add the mobile phase to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Sarpogrelate Hydroxide), add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> under the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sarpogrelate to that of the internal standard.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times Q_T/Q_S \times 5$

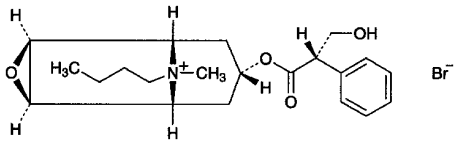
M_S: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sarpogrelate 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Scopolamine Butylbromide

プチルスコポラミン臭化物



$C_{21}H_{30}BrNO_4$: 440.37
(1*S*,2*S*,4*R*,5*R*,7*s*)-9-Butyl-7-[(2*S*)-3-hydroxy-2-phenylpropanoyloxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane bromide
[*149-64-4*]

Scopolamine Butylbromide, when dried, contains not less than 98.5% of $C_{21}H_{30}BrNO_4$.

Description Scopolamine Butylbromide occurs as white crystals or crystalline powder.
It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.
Melting point: about 140°C (with decomposition).

Identification **(1)** To 1 mg of Scopolamine Butylbromide add 3 to 4 drops of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.
(2) Determine the absorption spectrum of a solution of Scopolamine Butylbromide (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared absorption spectrum of Scopolamine Butylbromide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(4) A solution of Scopolamine Butylbromide (1 in 20) responds to the Qualitative Tests <*1.09*> for bromide.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -18.0 – $-20.0°$ (after drying, 1 g, water, 10 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the solution is clear, and has no more color than the following control solution.
Control solution: To 0.5 mL of Matching Fluid F add diluted hydrochloric acid (1 in 40) to make 20 mL.
(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Scopolamine Butylbromide 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) Related substances—Dissolve 0.10 g of Scopolamine Butylbromide in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of scopolamine hydrobromide in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Pipet 5 mL of the 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 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of scopolamine from the sample solution is not larger than that from the standard solution (2), and each area of the peaks other than the peak appearing in the first elution and the peak of scopolamine and butylscopolamine from the sample solution are not larger than the peak area from the standard solution (1).

Operating conditions—

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

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

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in 370 mL of water and 680 mL of methanol, and adjust the pH to 3.6 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust the flow rate so that the retention time of butylscopolamine is about 7 minutes.

Time span of measurement: About twice as long as the retention time of butylscopolamine.

System suitability—

System performance: Dissolve 5 mg each of Scopolamine Butylbromide and scopolamine hydrobromide in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, scopolamine and butylscopolamine 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 of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of scopolamine is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

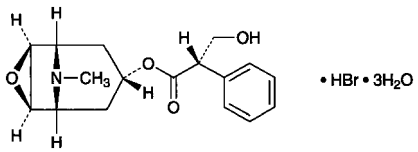
Assay Weigh accurately about 0.8 g of Scopolamine Butylbromide, previously dried, dissolve in 40 mL of acetic acid (100) and 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.04 mg of $C_{21}H_{30}BrNO_4$

Containers and storage Containers—Tight containers.

Scopolamine Hydrobromide Hydrate

スコポラミン臭化水素酸塩水和物



$C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31

(1*S*,2*S*,4*R*,5*R*,7*S*)-9-Methyl-3-oxa-9-azatricyclo-[3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate [*6533-68-2*]

Scopolamine Hydrobromide Hydrate, when dried, contains not less than 98.5% of scopolamine hydrobromide ($C_{17}H_{21}NO_4.HBr$: 384.26).

Description Scopolamine Hydrobromide Hydrate occurs as colorless or white crystals, or white granules or powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Identification (1) To 1 mg of Scopolamine Hydrobromide Hydrate add 3 to 4 drops of fuming nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced.

(2) A solution of Scopolamine Hydrobromide Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: −24.0 – −26.0° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point <2.60> 195 – 199°C (after drying; previously heat the bath to 180°C).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Scopolamine Hydrobromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.50 g of Scopolamine Hydrobromide Hydrate in 15 mL of water, and add 0.50 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Apoatropine—Dissolve 0.20 g of Scopolamine Hydrobromide Hydrate in 20 mL of water, add 0.60 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color in the solution does not disappear.

(4) Related substances—Dissolve 0.15 g of Scopolamine Hydrobromide Hydrate in 3 mL of water, and use this solution as the sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the sample solution add 2 to 3 drops of potassium hydroxide TS: a transient white turbidity might be produced, and disappears clearly in a little while.

Loss on drying ⟨*2.41*⟩ Not more than 13.0% [1.5 g; first dry in a desiccator (silica gel) for 24 hours, then dry at 105°C for 3 hours].

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

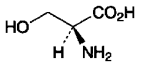
Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide Hydrate, previously dried, in 10 mL of acetic acid (100) by warming. After cooling, add 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.43 mg of $C_{17}H_{21}NO_4.HBr$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

L-Serine

L-セリン



$C_3H_7NO_3$: 105.09
(2*S*)-2-Amino-3-hydroxypropanoic acid
[*56-45-1*]

L-Serine, when dried, contains not less than 98.5% and not more than 101.0% of $C_3H_7NO_3$.

Description L-Serine occurs as white crystals or a crystalline powder. It has a slight sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in 2 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Serine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +14.0 – +16.0° (After drying, 2.5 g, 2 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.0 g of L-Serine in 10 mL of water is between 5.2 and 6.2.

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

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Serine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Serine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Serine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of L-Serine 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).

(6) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Serine according to Method 1, 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 L-Serine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, 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 Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop 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 evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.11 g of L-Serine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.51 mg of $C_3H_7NO_3$

Containers and storage Containers—Tight containers.

Serrapeptase

セラペプターゼ

[*95077-02-4*]

Serrapeptase is the enzyme preparation having proteolytic activity, produced by the growth of *Serratia* species.

Usually, it is diluted with Lactose Hydrate.

It contains not less than 2000 serrapeptase Units and not more than 2600 serrapeptase Units per mg.

It is hygroscopic.

Description Serrapeptase occurs as a grayish white to light brown powder, having a slight characteristic odor.

Identification Dissolve 0.4 g of Serrapeptase in 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0, transfer exactly 1 mL each of this solution into three tubes, and refer to them as A, B and C. To tube A add exactly 1 mL of water, to tubes B and C add exactly 1 mL of 0.04 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, mix gently, and allow them to stand in a water bath at 4 ± 1°C for about 1 hour. Then, to the tube B add exactly 2 mL of 0.04 mol/L zinc chloride TS, to the tubes A and C add ex-

actly 2 mL of water, mix gently, and allow them to stand in a water bath at 4 ± 1°C for about 1 hour. Pipet 1 mL each of these solutions, add borate-hydrochloric acid buffer solution, pH 9.0 to the solutions A and B to make exactly 200 mL, to the solution C to make exactly 50 mL, and use these solutions as the sample solutions. Proceed with these sample solutions as directed in the Assay: the activities of the solutions A and B are almost the same, and the activity of the solution C is not more than 5% of that of the solution A.

Activity of solutions A, B or C = $A_T/A_S \times 1/20 \times D \times 176$

A_S: Absorbance of the standard solution
A_T: Absorbance of the sample solution
20: Reaction time (minute)
D: Dilution rate (200 for solution A and B, 50 for solution C)
176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken × amount of tyrosine in 2 mL of tyrosine standard solution)

Purity **(1)** Heavy metals <*1.07*>—Put 1.0 g of Serrapeptase in a porcelain crucible, add 2 drops each of sulfuric acid and nitric acid, and incinerate by ignition. After cooling, to the residue add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, add 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. After cooling, filter if necessary, wash the filter paper with 10 mL of water, put the filtrate and washing in a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: Evaporate to dryness 2 drops each of sulfuric acid and nitric acid on a sand bath, add 2 mL of hydrochloric acid to the residue, evaporate to dryness on a water bath, add 2.0 mL of Standard Lead Solution, 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. Proceed in the same manner as directed for the preparation of the test solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Serrapeptase according to Method 3, excepting addition of 5 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (3 in 10) instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), evaporating to dryness on a water bath, then incinerating with a small flame, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 1.5% (1 g).

Assay (i) Sample solution: Dissolve exactly 0.100 g of Serrapeptase in a solution of ammonium sulfate (1 in 20) to make exactly 100 mL. Pipet 1 mL of this solution, add borate-hydrochloric acid buffer solution, pH 9.0 to make exactly 200 mL, and use this solution as the sample solution.

(ii) Tyrosine standard solution: Dissolve exactly 0.160 g of Tyrosine RS, previously dried at 105°C for 3 hours, in 0.2 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet 10 mL of this solution, and add 0.2 mol/L hydrochloric acid TS to make exactly 100 mL. Prepare before use.

(iii) Substrate solution: Previously determine the loss on drying <*2.41*> (60°C, reduced pressure not exceeding 0.67 kPa, 3 hours) of milk casein, previously dried. To exactly 1.20 g of the milk casein, calculated based on the loss on drying, add 160 mL of a solution of sodium borate (19 in 1000), and heat in a water bath to dissolve. After cooling, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add borate-hydrochloric acid buffer solution, pH 9.0 to make exactly 200 mL. Use after warming to 37 ± 0.5°C. Prepare before use.

(iv) Precipitation reagent: Trichloroacetic acid TS for serrapeptase. Use after warming to 37 ± 0.5°C.

(v) Procedure: Pipet 1 mL of the sample solution, put in a glass-stoppered tube (15 × 130 mm), allow to stand at 37 ± 0.5°C for 5 minutes, add exactly 5 mL of the substrate solution, and mix well immediately. Allow to stand at 37 ± 0.5°C for exactly 20 minutes, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, allow to stand at 37 ± 0.5°C for 30 minutes, and filter through a dried filter paper. Pipet 2 mL of the filtrate, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and allow to stand at 37 ± 0.5°C for 30 minutes. Determine the absorbance of this solution at 660 nm, A_1, as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, add exactly 5 mL of the substrate solution, allow to stand at 37 ± 0.5°C for 30 minutes, and proceed in the same manner as directed above to determine the absorbance A_2. Separately, pipet 2 mL of the tyrosine standard solution, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and proceed in the same manner as directed above to determine the absorbance A_3. Separately, pipet 2 mL of 0.2 mol/L hydrochloric acid TS, and proceed in the same manner as directed above to determine the absorbance A_4.

Serrapeptase Unit per mg of Serrapeptase
$= (A_1 - A_2)/(A_3 - A_4) \times 1/20 \times 200 \times 176$

20: Reaction time (minute)
200: Dilution rate
176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken × amount of tyrosine in 2 mL of tyrosine standard solution)

One serrapeptase Unit corresponds to the amount of serrapeptase which produces 1 μg of tyrosine per minute from 5 mL of the substrate solution under the above conditions.

Containers and storage Containers—Tight containers.

Sesame Oil

Oleum Sesami

ゴマ油

Sesame Oil is the fixed oil obtained from the seeds of *Sesamum indicum* Linné (*Pedaliaceae*).

Description Sesame Oil is a clear, pale yellow oil. It is odorless or has a faint, characteristic odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95).

It congeals between 0°C and −5°C.
Congealing point of the fatty acids: 20 – 25°C

Identification To 1 mL of Sesame Oil add 0.1 g of sucrose and 10 mL of hydrochloric acid, and shake for 30 seconds: the acid layer becomes light red and changes to red on standing.

Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 0.914 – 0.921

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 187 – 194

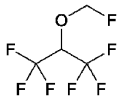
Unsaponifiable matters ⟨*1.13*⟩ Not more than 2.0%.

Iodine value ⟨*1.13*⟩ 103 – 118

Containers and storage Containers—Tight containers.

Sevoflurane

セボフルラン



$C_4H_3F_7O$: 200.05
1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane
[*28523-86-6*]

Sevoflurane contains not less than 99.0% and not more than 101.0% of $C_4H_3F_7O$, calculated on the anhydrous basis.

Description Sevoflurane is a clear, colorless, and mobile liquid.
It is miscible with ethanol (99.5).
It is very slightly soluble in water.
It is volatile and inflammable.
Refractive index n_D^{20}: 1.2745 – 1.2760
Boiling point: about 58.6°C.

Identification Transfer about 1 μL of Sevoflurane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Sevoflurane RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.510 – 1.530

Purity **(1)** Acidity or alkalinity—To 50 mL of Sevoflurane with 50 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer and use this solution as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Soluble fluoride—To 6 g of Sevoflurane 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 diluted 0.01 mol/L sodium hydroxide solution (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 sample solution. Separately, transfer 0.2 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) into a Nessler tube, and 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). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 1 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).

(3) Related substances—Perform the test with 2 μL of Sevoflurane as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of them by the area percentage method: the amount of the peak of hexafluoroisopropyl methyl ether, having the relative retention time of about 0.84 with respect to sevoflurane, is not more than 0.005%, the amount of each peak other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.0025%, and the total amount of the peaks other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.005%.
Operating conditions—
Detector, column, injection port temperature, detector temperature, carrier gas and split ratio: Proceed as directed in the operating conditions in the Assay.
Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 10 minutes, raise at a rate of 10°C per minute to 200°C, and maintain at a constant temperature of about 200°C.
Flow rate: Adjust the flow rate so that the retention time of sevoflurane is about 7 minutes.
Time span of measurement: About 6 times as long as the retention time of sevoflurane.
System suitability—
Test for required detectability: To 20 μL of Sevoflurane add *o*-xylene to make 20 mL. To 1 mL of this solution add *o*-xylene to make 20 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test and add *o*-xylene to make exactly 10 mL. Confirm that the peak area of sevoflurane obtained from 2 μL of this solution is equivalent to 7 to 13% of the peak area of sevoflurane from 2 μL of the solution for system suitability test.
System performance: When the procedure is run with 2 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of sevoflurane are not less than 6000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 2 μL of the solution for system suitability test under the

above operating conditions, the relative standard deviation of the peak area of Sevoflurane is not more than 5.0%.

(4) Residual solvent—Being specified separately.

(5) Residue on evaporation—Evaporate 10 mL of Sevoflurane, exactly measured, on a water bath to dryness, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.

Water ⟨*2.48*⟩ Not more than 0.2 w/v% (5 mL, volumetric titration, direct titration).

Assay Pipet 5 mL each of Sevoflurane and Sevoflurane RS (separately determine the water ⟨*2.48*⟩ using the same manner as Sevoflurane), to each add exactly 5 mL of dimethoxymethane as an internal standard, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sevoflurane to that of the internal standard.

$$\text{Amount (mg) of sevoflurane } (C_4H_3F_7O) = V_S \times Q_T/Q_S \times 1000 \times 1.521$$

V_S: Amount (mL) of Sevoflurane RS, calculated on the anhydrous basis

1.521: Specific gravity of Sevoflurane (d_{20}^{20})

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with cyanopropyl methylphenyl silicone for gas chromatography in 1.8 μm thickness.

Column temperature: 40°C.

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 225°C.

Carrier gas: Helium.

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

Split ratio: 1:20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, sevoflurane 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sevoflurane to that of the internal standard is not more than 1.0 %.

Containers and storage Containers—Tight containers.

Purified Shellac

精製セラック

Purified Shellac is a resin-like substance obtained from a purified secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description Purified Shellac occurs as light yellow-brown to brown, lustrous, hard, brittle scutella. It has no odor or has a faint, characteristic odor.

It is freely soluble in ethanol (95) and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Acid value ⟨*1.13*⟩ 60 – 80 Weigh accurately about 1 g of Purified Shellac, add 40 mL of neutralized ethanol, and dissolve by warming. After cooling, titrate ⟨*2.50*⟩ with 0.1 mol/L potassium hydroxide VS (potentiometric titration).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Purified Shellac 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 ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Purified Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(3) Ethanol-insoluble substances—Dissolve about 5 g of Purified Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at 105°C for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(4) Rosin—Dissolve 2.0 g of Purified Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(5) Wax—Dissolve 10.0 g of Purified Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, ad dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 2.0%. Weigh accurately about 1 g of medium powder of Purified Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

Total ash <*5.01*> Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

White Shellac

白色セラック

White Shellac is a resin-like substance obtained from a bleached secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description White Shellac occurs as yellowish white to light yellow, hard, brittle granules. It is odorless or has a faint, characteristic odor.

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

It dissolves in sodium hydroxide TS.

Acid value <*1.13*> 65 – 90 Weigh accurately about 0.5 g of White Shellac, add 50 mL of neutralized ethanol as a solvent, and dissolve by warming. After cooling, perform the test as directed in the Acid value under Purified Shellac.

Purity **(1)** Chloride <*1.03*>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) while warming, add 40 mL of water, and cool. Add 12 mL of dilute nitric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.80 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.140%).

(2) Sulfate <*1.14*>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) by warming, add 40 mL of water, and cool. Add 2 mL of dilute hydrochloric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.005 mol/L sulfuric acid VS add 2.5 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.110%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of White Shellac 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 <*1.11*>—Prepare the test solution with 0.40 g of White Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(5) Ethanol-insoluble substances—Dissolve about 5 g of White Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at 105°C for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(6) Rosin—Dissolve 2.0 g of White Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(7) Wax—Dissolve 10.0 g of White Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 6.0%. Weigh accurately about 1 g of medium powder of White Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

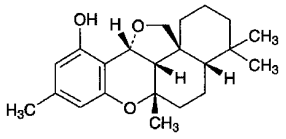
Total ash <*5.01*> Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

Storage—In a cold place.

Siccanin

シッカニン



$C_{22}H_{30}O_3$: 342.47

(4a*S*,6a*S*,11b*R*,13a*S*,13b*S*)-4,4,6a,9-Tetramethyl-1,2,3,4,4a,5,6,6a,11b,13b-decahydro-13*H*-benzo[*a*]furo[2,3,4-*mn*]xanthen-11-ol

[*22733-60-4*]

Siccanin is a substance having antifungal activity produced by the growth of *Helminthosporium siccans*.

It contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Siccanin is expressed as mass (potency) of siccanin ($C_{22}H_{30}O_3$).

Description Siccanin occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a

solution of Siccanin in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Siccanin RS obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Siccanin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Siccanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -165 – $-175°$ (0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> 138 – 142°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Siccanin 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 0.20 g of Siccanin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone 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 <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-chlorobenzenediazonium TS on the plate: the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Siccanin and Siccanin RS, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of siccanin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{22}H_{30}O_3 = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Siccanin RS

Internal standard solution—A solution of 1,4-diphenylbenzene in methanol (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside 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 phosphate buffer solution, pH 5.9 (19:6).

Flow rate: Adjust the flow rate so that the retention time of siccanin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, siccanin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of siccanin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Light Anhydrous Silicic Acid

軽質無水ケイ酸

Light Anhydrous Silicic Acid, calculated on the incinerated basis, contains not less than 98.0% of silicon dioxide (SiO_2: 60.08).

Description Light Anhydrous Silicic Acid occurs as a white to bluish white, light, fine power. It is odorless and tasteless, and smooth to the touch.

It is practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in hydrofluoric acid, in hot potassium hydroxide TS and in hot sodium hydroxide TS, and does not dissolve in dilute hydrochloric acid.

Identification **(1)** Dissolve 0.1 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, and add 12 mL of ammonium chloride TS: a white, gelatinous precipitate is produced. The precipitate does not dissolve in dilute hydrochloric acid.

(2) To the precipitate obtained in (1) add 10 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with water: the precipitate has a blue color.

(3) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Bring the hot, transparent bead into contact with Light Anhydrous Silicic Acid, and fuse again: an insoluble matter is perceptible in the bead. The resulting bead, upon cooling, becomes opaque and acquires a reticulated appearance.

Purity **(1)** Chloride <1.03>—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, filter if necessary, and wash with 10 mL of water. Combine the filtrate and washings, add 18 mL of dilute nitric acid, shake, and add water to make 50 mL. Perform the test using this solution as the test solution. To 0.15 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of sodium hydroxide TS, 18 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution (not more than 0.011%).

(2) Heavy metals <1.07>—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, add 15 mL of acetic acid (31), shake, filter if nec-

essary, wash with 10 mL of water, combine the filtrate and washings, and add water to make 50 mL. Perform the test using this solution as the test solution. Add acetic acid (31) to 20 mL of sodium hydroxide TS and 1 drop of phenolphthalein TS until the color of this solution disappears, add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 40 ppm).

(3) Iron <*1.10*>—To 40 mg of Light Anhydrous Silicic Acid add 10 mL of dilute hydrochloric acid, and heat for 10 minutes in a water bath while shaking. After cooling, add 0.5 g of L-tartaric acid to dissolve by shaking. Prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(4) Aluminum—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 40 mL of sodium hydroxide TS by boiling, cool, add sodium hydroxide TS to make 50 mL, and filter. Measure 10 mL of the filtrate, add 17 mL of acetic acid (31), shake, add 2 mL of aluminon TS and water to make 50 mL, and allow to stand for 30 minutes: the color of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.176 g of aluminum potassium sulfate dodecahydrate in water, and add water to make 1000 mL. To 15.5 mL of this solution add 10 mL of sodium hydroxide TS, 17 mL of acetic acid (31), 2 mL of aluminon TS and water to make 50 mL.

(5) Calcium—Dissolve 1.0 g of Light Anhydrous Silicic Acid in 30 mL of sodium hydroxide TS by boiling, cool, add 20 mL of water, 1 drop of phenolphthalein TS and dilute nitric acid until the color of this solution disappears, immediately add 5 mL of dilute acetic acid, shake, add water to make 100 mL, and obtain a clear liquid by centrifugation or filtration. To 25 mL of this liquid add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, immediately shake, and allow to stand for 10 minutes: the turbidity of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.250 g of calcium carbonate, previously dried at 180°C for 4 hours, in 3 mL of dilute hydrochloric acid, and add water to make 100 mL. To 4 mL of this solution add 5 mL of dilute acetic acid and water to make 100 mL. To 25 mL of this solution add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, and shake.

(6) Arsenic <*1.11*>—Dissolve 0.40 g of Light Anhydrous Silicic Acid in 10 mL of sodium hydroxide TS by boiling in a porcelain crucible, cool, add 5 mL of water and 5 mL of dilute hydrochloric acid, shake, and perform the test with this solution as the test solution (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C, 4 hours).

Loss on ignition <*2.43*> Not more than 12.0% (1 g, 850 – 900°C, constant mass).

Volume test Weigh 5.0 g of Light Anhydrous Silicic Acid, transfer gradually to a 200-mL measuring cylinder, and allow to stand: the volume is not less than 70 mL.

Assay Weigh accurately about 1 g of Light Anhydrous Silicic Acid, add 20 mL of hydrochloric acid, and evaporate to dryness on a sand bath. Moisten the residue with hydrochloric acid, evaporate to dryness, and heat between 110°C and 120°C for 2 hours. Cool, add 5 mL of dilute hydrochloric acid, and heat. Allow to cool to room temperature, add 20 to 25 mL of hot water, filter rapidly, and wash the residue with warm water until the last washing becomes negative to the Qualitative Tests <*1.09*> (2) for chloride. Transfer the residue together with the filter paper to a platinum crucible, ignite to ash, and continue the ignition for 30 minutes. Cool, weigh the crucible, and designate the mass as a (g). Moisten the residue in the crucible with water, add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid, and evaporate to dryness. Heat strongly for 5 minutes, cool, weigh the crucible, and designate the mass as b (g).

$$\text{Content (g) of silicon dioxide } (SiO_2) = a - b$$

Containers and storage Containers—Tight containers.

Silver Nitrate

硝酸銀

$AgNO_3$: 169.87

Silver Nitrate, when dried, contains not less than 99.8% of $AgNO_3$.

Description Silver Nitrate occurs as lustrous, colorless or white crystals.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually turns grayish black by light.

Identification A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests <*1.09*> for silver salt and for nitrate.

Purity **(1)** Clarity and color of solution, and acidity or alkalinity—Dissolve 1.0 g of Silver Nitrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless. It is neutral.

(2) Bismuth, copper and lead—To 5 mL of a solution of Silver Nitrate (1 in 10) add 3 mL of ammonia TS: the solution is clear and colorless.

Loss on drying <*2.41*> Not more than 0.20% (2 g, silica gel, light resistant, 4 hours).

Assay Weigh accurately about 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid, and titrate <*2.50*> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

$$\text{Each mL of 0.1 mol/L ammonium thiocyanate VS} = 16.99 \text{ mg of } AgNO_3$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Nitrate Ophthalmic Solution

硝酸銀点眼液

Silver Nitrate Ophthalmic Solution is an aqueous eye lotion.

It contains not less than 0.95 w/v% and not more than 1.05 w/v% of silver nitrate ($AgNO_3$: 169.87).

Method of preparation

Silver Nitrate	10 g
Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Ophthalmic Preparations, with the above ingredients.

Description Silver Nitrate Ophthalmic Solution is a clear, colorless liquid.

Identification Silver Nitrate Ophthalmic Solution responds to the Qualitative Tests <*1.09*> for silver salt and for nitrate.

Assay Measure accurately 20 mL of Silver Nitrate Ophthalmic Solution, add 30 mL of water and 2 mL of nitric acid, and titrate <*2.50*> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 16.99 mg of $AgNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Protein

プロテイン銀

Silver Protein is a compound of silver and proteins.

It contains not less than 7.5% and not more than 8.5% of silver (Ag: 107.87).

Description Silver Protein occurs as a light yellow-brown to brown powder. It is odorless.

It (1 g) dissolves slowly in 2 mL of water. It is practically insoluble in ethanol (95), in diethyl ether and in chloroform.

The pH of a solution of Silver Protein (1 in 10) is between 7.0 and 8.5.

It is slightly hygroscopic.

It is affected by light.

Identification **(1)** To 10 mL of a solution of Silver Protein (1 in 100) add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. To the filtrate add 5 mL of a solution of sodium hydroxide (1 in 10), and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops.

(2) To 5 mL of a solution of Silver Protein (1 in 100) add dropwise iron (III) chloride TS: the color of the solution fades and a precipitate is gradually formed.

(3) Incinerate 0.2 g of Silver Protein by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: this solution responds to the Qualitative Tests <*1.09*> (1) for silver salt.

Purity Silver salt—Dissolve 0.10 g of Silver Protein in 10 mL of water, and filter. To the filtrate add 1 mL of potassium chromate TS: no turbidity is produced.

Assay Transfer about 1 g of Silver Protein, accurately weighed, to a 100-mL decomposition flask, add 10 mL of sulfuric acid, cover the flask with a funnel, and boil for 5 minutes. Cool, add dropwise 3 mL of nitric acid with caution, and heat for 30 minutes without boiling. Cool, add 1 mL of nitric acid, boil, and, if necessary, repeat this operation until the solution becomes colorless. After cooling, transfer the solution to a 250-mL conical flask with 100 mL of water, and titrate <*2.50*> with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 10.79 mg of Ag

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Protein Solution

プロテイン銀液

Silver Protein Solution contains not less than 0.22 w/v% and not more than 0.26 w/v% of silver (Ag: 107.87).

Method of preparation

Silver Protein	30 g
Glycerin	100 mL
Mentha Water	a sufficient quantity
To make	1000 mL

Dissolve and mix the above ingredients.

Description Silver Protein Solution is a clear, brown liquid, having the odor of mentha oil.

Identification **(1)** To 1 mL of Silver Protein Solution add 10 mL of ethanol (95), mix, and add 2 mL of sodium hydroxide TS. Add immediately 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), shake, and filter: the filtrate is blue in color (glycerin).

(2) To 3 mL of Silver Protein Solution add water to make 10 mL, add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. Add 5 mL of a solution of sodium hydroxide (1 in 10) to the filtrate, and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops (silver protein).

(3) To 5 mL of the sample solution obtained in (2) add iron (III) chloride TS dropwise: a brown precipitate is formed (silver protein).

(4) Place 3 mL of Silver Protein Solution in a crucible, heat cautiously, and evaporate almost to dryness. Then incinerate gradually by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: the solution responds to the Qualitative Tests <*1.09*> (1) for silver salt.

Assay Pipet 25 mL of Silver Protein Solution into a 250-mL Kjeldahl flask, and heat cautiously until a white gas of glycerin is evolved. After cooling, add 25 mL of sulfuric acid, cover the flask with a funnel, and heat gently for 5 minutes. After cooling, drop gradually 5 mL of nitric acid, heat with occasional shaking in a water bath for 45 minutes, and cool. Add 2 mL of nitric acid, boil gently, and repeat this operation until the solution becomes colorless upon cooling. Transfer cautiously the cooled content in the flask into a 500-mL conical flask with 250 mL of water. Boil gently for 5 minutes, cool, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 10.79 mg of Ag

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Simple Ointment

単軟膏

Method of preparation

Yellow Beeswax	330 g
Fixed oil	a sufficient quantity
To make	1000 g

Prepare as directed under Ointments, with the above ingredients.

Description Simple Ointment is yellow in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Simple Syrup

単シロップ

Simple Syrup is an aqueous solution of Sucrose.

Method of preparation

Sucrose	850 g
Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Syrups, with the above materials.

Description Simple Syrup is a clear, colorless to pale yellow, viscous liquid. It is odorless and has a sweet taste.

Identification **(1)** Evaporate Simple Syrup on a water bath to dryness. 1 g of the residue so obtained, when ignited, melts to swell, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of the residue obtained in (1) add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark red precipitate is produced.

Specific gravity <2.56> d_{20}^{20}: 1.310 – 1.325

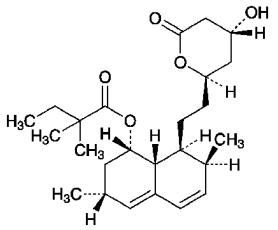
Purity **(1)** Artificial sweetening agents—To 100 mL of Simple Syrup add 100 mL of water, shake, acidify a 50-mL portion of the solution with dilute sulfuric acid, and make another 50-mL portion alkaline with sodium hydroxide TS. To each portion add 100 mL of diethyl ether, shake, separate the diethyl ether layer, and evaporate the combined diethyl ether extract on a water bath to dryness: the residue has no sweet taste.

(2) Salicylic acid—To the residue obtained in (1) add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Containers and storage Containers—Tight containers.

Simvastatin

シンバスタチン



$C_{25}H_{38}O_5$: 418.57
(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate
[*79902-63-9*]

Simvastatin contains not less than 98.0% and not more than 101.0% of $C_{25}H_{38}O_5$, calculated on the dried basis.

It may contain a suitable antioxidant.

Description Simvastatin occurs as a white, crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Simvastatin in acetonitrile (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Simvastatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Simvastatin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Simvastatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +285 – +300° (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1 g of

Simvastatin in 10 mL of methanol: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 440 nm is not more than 0.10.

(2) Heavy metals <1.07>—To 1.0 g of Simvastatin add 2 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 0.5 mL of nitric acid, heat in the same manner as above, and ignite at 500 to 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to 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 20 ppm).

(3) Related substances—Dissolve 30 mg of Simvastatin in 20 mL of a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amounts of the peaks, having the relative retention times of about 0.45, about 0.80, about 2.42, and about 3.80 with respect to simvastatin are not more than 0.2%, respectively; the amount of the peak with a relative retention time of about 2.38 is not more than 0.3%; the amount of the peak with a relative retention time of about 0.60 is not more than 0.4%; and the amount of each peak other than simvastatin and other than the peaks mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than simvastatin and other than the peak with relative retention time of about 0.60 with respect to simvastatin is not more than 1.0%.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).

Mobile phase B: A solution of phosphoric acid in acetonitrile for liquid chromatography (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 4.5	100	0
4.5 – 4.6	100 → 95	0 → 5
4.6 – 8.0	95 → 25	5 → 75
8.0 – 11.5	25	75

Flow rate: 3.0 mL per minute.

Time span of measurement: About 5 times as long as the retention time of simvastatin.

System suitability—

Test for required detectability: To 0.5 mL of the sample solution, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 10 mL. Confirm that the peak area of simvastatin obtained from 5 μL of this solution is equivalent to 16 to 24% of the peak area of simvastatin from 5 μL of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Simvastatin and Simvastatin RS (previously determine the loss on drying <2.41> under the same conditions as Simvastatin), dissolve each in a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of simvastatin for each solution.

Amount (mg) of simvastatin ($C_{25}H_{38}O_5$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 33 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: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).

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

System suitability—

System performance: Dissolve 3 mg of lovastatin in 2 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, lovastatin and simvastatin 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Under nitrogen atmosphere.

Freeze-dried Smallpox Vaccine

乾燥痘そうワクチン

Freeze-dried Smallpox Vaccine is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine becomes a white to gray, turbid liquid on addition of solvent.

Freeze-dried Smallpox Vaccine Prepared in Cell Culture

乾燥細胞培養痘そうワクチン

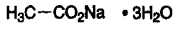
Freeze-dried Smallpox Vaccine Prepared in Cell Culture is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine Prepared in Cell Culture in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine Prepared in Cell Culture becomes a reddish clear liquid on addition of solvent.

Sodium Acetate Hydrate

酢酸ナトリウム水和物



$C_2H_3NaO_2.3H_2O$: 136.08
Monosodium acetate trihydrate
[*6131-90-4*]

Sodium Acetate Hydrate, when dried, contains not less than 99.5% of sodium acetate ($C_2H_3NaO_2$: 82.03).

Description Sodium Acetate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless or has a slight, acetous odor. It has a cool, saline and slightly bitter taste.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), and practically insoluble in diethyl ether.

It is efflorescent in warm, dry air.

Identification A solution of Sodium Acetate Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for acetate and for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Sodium Acetate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Sodium Acetate Hydrate in 20 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: a red color develops. When cooled to 10°C, or 1.0 mL of 0.01 mol/L hydrochloric acid VS is added after cooling to 10°C, the red color disappears.

(3) Chloride <*1.03*>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(4) Sulfate <*1.14*>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Acetate 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).

(6) Calcium and magnesium—Dissolve 4.0 g of Sodium Acetate Hydrate in 25 mL of water, add 6 g of ammonium chloride, 20 mL of ammonia solution (28) and 0.25 mL of a solution of sodium sulfite heptahydrate (1 in 10), and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue color changes to grayish blue (indicator: 0.1 g of methylthymol blue-potassium nitrate indicator): the amount of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed is not more than 0.5 mL.

(7) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Sodium Acetate Hydrate, according to Method 1, and perform the test (not more than 2 ppm).

(8) Potassium permanganate-reducing substance—Dissolve 1.0 g of Sodium Acetate Hydrate in 100 mL of water, add 5 mL of dilute sulfuric acid, boil, add 0.50 mL of 0.002 mol/L potassium permanganate VS, and further boil for 5 minutes: the red color of the solution does not disappear.

Loss on drying <*2.41*> 39.0 – 40.5% (1 g, first at 80°C for 2 hours, and then at 130°C for 2 hours).

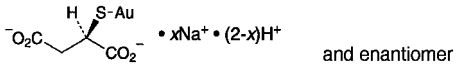
Assay Weigh accurately about 0.2 g of Sodium Acetate Hydrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 1 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.203 mg of $C_2H_3NaO_2$

Containers and storage Containers—Tight containers.

Sodium Aurothiomalate

金チオリンゴ酸ナトリウム



and enantiomer

Mixture of $C_4H_3AuNa_2O_4S$: 390.08 and $C_4H_4AuNaO_4S$: 368.09
Monogold monosodium monohydrogen (2*RS*)-2-sulfidobutane-1,4-dioate
Monogold disodium (2*RS*)-2-sulfidobutane-1,4-dioate
[*12244-57-4*, Sodium Aurothiomalate]

Sodium Aurothiomalate contains not less than 49.0% and not more than 52.5% of gold (Au: 196.97), calculated on the anhydrous basis and corrected by the amount of ethanol.

Description Sodium Aurothiomalate occurs as white to light yellow, powder or granules.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

It changes in color by light to greenish pale yellow.

Identification **(1)** To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 1 mL of a solution of calcium nitrate tetrahydrate (1 in 10): a white precipitate is produced, and it dissolves in dilute nitric acid and reappears on the addition of ammonium acetate TS.

(2) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 3 mL of silver nitrate TS: a yellow precipitate is produced, and it dissolves in an excess of ammonia TS.

(3) Place 2 mL of a solution of Sodium Aurothiomalate (1 in 10) in a porcelain crucible, add 1 mL of ammonia TS and 1 mL of hydrogen peroxide (30), evaporate to dryness, and ignite. Add 20 mL of water to the residue, and filter: the residue on the filter paper occurs as a yellow or dark yellow, powder or granules.

(4) The filtrate obtained in (3) responds to the Qualitative Tests <*1.09*> for sodium salt.

(5) The filtrate obtained in (3) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 1.0 g of Sodium Aurothiomalate in 10 mL of water: the pH of this solution is between 5.8 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Aurothiomalate in 10 mL of water: the solution is clear and light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Sodium Aurothiomalate 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 <*1.11*>—Prepare the test solution with 1.0 g of Sodium Aurothiomalate according to Method 3, and perform the test (not more than 2 ppm).

(4) Ethanol—Weigh accurately about 0.2 g of Sodium Aurothiomalate, add exactly 3 mL of the internal standard solution and 2 mL of water to dissolve, and use this solution as the sample solution. Separately, pipet 3 mL of ethanol (99.5), and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios of the peak area of ethanol to that of the internal standard, Q_T and Q_S: the amount of ethanol is not more than 3.0%.

$$\text{Amount (mg) of ethanol} = Q_T/Q_S \times 6 \times 0.793$$

0.793: Density (g/mL) of ethanol (99.5) at 20°C

Internal standard solution—A solution of 2-propanol (1 in 500).

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (particle diameter: 150 – 180 μm) (average pore size: 0.0085 μm; 300 – 400 m^2/g).

Column temperature: A constant temperature of about 180°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard 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, ethanol 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 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water <*2.48*> Not more than 5.0% (0.1 g, coulometric titration). Use a water vaporizer (heating temperature: 105°C; heating time: 30 minutes).

Assay Weigh accurately about 25 mg of Sodium Aurothiomalate, and dissolve in 2 mL of aqua regia by heating. After cooling, add water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet 5 mL, 10 mL and 15 mL of Standard Gold Solution for atomic absorption spectrophotometry, add water to make exactly 25 mL, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with the sample solution and standard solutions (1), (2) and (3) as directed under Atomic Absorption Spectrophotometry <*2.23*> under the following conditions. Determine the amount of gold in the sample solution using the calibration curve obtained from the absorbances of the standard solutions.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.

Lamp: Gold hollow-cathode lamp.

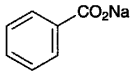
Wavelength: 242.8 nm.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sodium Benzoate

安息香酸ナトリウム



$C_7H_5NaO_2$: 144.10
Monosodium benzoate
[*532-32-1*]

Sodium Benzoate, when dried, contains not less than 99.0% of $C_7H_5NaO_2$.

Description Sodium Benzoate occurs as white granules, crystals or crystalline powder. It is odorless, and has a sweet and saline taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification A solution of Sodium Benzoate (1 in 100) responds to the Qualitative Tests <*1.09*> for benzoate and the Qualitative Tests <*1.09*> (1) and (2) for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Benzoate in 20 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS: the solution remains colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Sulfate <*1.14*>—Dissolve 0.40 g of Sodium Benzoate in 40 mL of water, add slowly 3.5 mL of dilute hydrochloric acid with thorough stirring, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, take the subsequent 20 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 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.120%).

(4) Heavy metals <*1.07*>—Dissolve 2.0 g of Sodium Benzoate in 44 mL of water, add gradually 6 mL of dilute hydrochloric acid with thorough stirring, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, neutralize with ammonia TS, 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 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).

(5) Arsenic <*1.11*>—Mix well 1.0 g of Sodium Benzoate with 0.40 g of calcium hydroxide, ignite, dissolve the residue in 10 mL of dilute hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

(6) Chlorinated compounds—Dissolve 1.0 g of Sodium Benzoate in 10 mL of water, add 10 mL of dilute sulfuric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate the diethyl ether on a water bath. Place 0.5 g of the residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, 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.

(7) Phthalic acid—To 0.10 g of Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

Loss on drying <*2.41*> Not more than 1.5% (2 g, 110°C, 4 hours).

Assay Weigh accurately about 1.5 g of Sodium Benzoate, previously dried, and transfer to a 300-mL glass-stoppered flask. Dissolve in 25 mL of water, add 75 mL of diethyl ether and 10 drops of bromophenol blue TS, and titrate <*2.50*> with 0.5 mol/L hydrochloric acid VS, while mixing the aqueous and diethyl ether layers by vigorous shaking, until a persistent, light green color is produced in the aqueous layer.

Each mL of 0.5 mol/L hydrochloric acid VS
= 72.05 mg of $C_7H_5NaO_2$

Containers and storage Containers—Well-closed containers.

Sodium Bicarbonate

Sodium Hydrogen Carbonate

炭酸水素ナトリウム

$NaHCO_3$: 84.01

Sodium Bicarbonate contains not less than 99.0% of $NaHCO_3$.

Description Sodium Bicarbonate occurs as white crystals or crystalline powder. It is odorless, and has a characteristic, saline taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It slowly decomposes in moist air.

Identification A solution of Sodium Bicarbonate (1 in 30) responds to the Qualitative Tests <*1.09*> for sodium salt and for bicarbonate.

pH <*2.54*> Dissolve 1.0 g of Sodium Bicarbonate in 20 mL

of water: the pH of this solution is between 7.9 and 8.4.

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

(2) Chloride <*1.03*>—To 0.40 g of Sodium Bicarbonate add 4 mL of dilute nitric acid, heat to boil, cool, 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 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040%).

(3) Carbonate—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of freshly boiled and cooled water with very gentle swirling at a temperature not exceeding 15°C. Add 2.0 mL of 0.1 mol/L hydrochloric acid VS and 2 drops of phenolphthalein TS: no red color develops immediately.

(4) Ammonium—Heat 1.0 g of Sodium Bicarbonate: the gas evolved does not change moistened red litmus paper to blue.

(5) Heavy metals <*1.07*>—Dissolve 4.0 g of Sodium Bicarbonate in 5 mL of water and 4.5 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid, 35 mL of water and 1 drop of ammonium TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 4.5 mL of hydrochloric acid to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(6) Arsenic <*1.11*>—Dissolve 1.0 g of Sodium Bicarbonate in 3 mL of water and 2 mL of hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, dissolve in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS. When the color of the solution changes from blue to yellow-green, boil with caution, cool, and continue the titration <*2.50*> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 84.01 mg of $NaHCO_3$

Containers and storage Containers—Tight containers.

Sodium Bicarbonate Injection

炭酸水素ナトリウム注射液

Sodium Bicarbonate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium hydrogen carbonate ($NaHCO_3$: 84.01).

Method of preparation Prepare as directed under Injections, with Sodium Bicarbonate.

Description Sodium Bicarbonate Injection is a clear, colorless liquid.

Identification To a volume of Sodium Bicarbonate Injection, equivalent to 1 g of Sodium Bicarbonate according to the labeled amount, add water to make 30 mL: the solution responds to the Qualitative Tests <*1.09*> for sodium salt and for bicarbonate.

pH <*2.54*> 7.0 – 8.5

Bacterial endotoxins <*4.01*> Less than 5.0 EU/mEq.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Sodium Bicarbonate Injection, equivalent to about 2 g of sodium hydrogen carbonate ($NaHCO_3$), titrate with 0.5 mol/L sulfuric acid VS, and proceed as directed in the Assay under Sodium Bicarbonate.

Each mL of 0.5 mol/L sulfuric acid VS
= 84.01 mg of $NaHCO_3$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Bisulfite

Sodium Hydrogen Sulfite

亜硫酸水素ナトリウム

$NaHSO_3$: 104.06

Sodium Bisulfite is a mixture of sodium hydrogensulfite and sodium pyrosulfite.

It contains not less than 64.0% and not more than 67.4% of sulfur dioxide (SO_2: 64.06).

Description Sodium Bisulfite occurs as white granules or powder, having the odor of sulfur dioxide.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Bisulfite (1 in 20) is acid.

It is slowly affected by air or by light.

Identification A solution of Sodium Bisulfite (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for bisulfite.

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

(2) Thiosulfate—Dissolve 1.0 g of Sodium Bisulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water, add 5 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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: evaporate 5 mL of hy-

drochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 1.0 g of Sodium Bisulfite according to Method 1, 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) Arsenic <*1.11*>—Dissolve 0.5 g of Sodium Bisulfite in 10 mL of water. Add 1 mL of sulfuric acid, heat on a sand bath until white fumes are evolved, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Bisulfite, and transfer immediately into an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate <*2.50*> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 3.203 mg of SO_2

Containers and storage Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Borate

ホウ砂

$Na_2B_4O_7.10H_2O$: 381.37

Sodium Borate contains not less than 99.0% and not more than 103.0% of $Na_2B_4O_7.10H_2O$.

Description Sodium Borate occurs as colorless or white crystals or a white, crystalline powder. It is odorless, and has a slightly characteristic, saline taste.

It is freely soluble in glycerin, soluble in water, and practically insoluble in ethanol (95), in ethanol (99.5) and in diethyl ether.

When placed in dry air, Sodium Borate effloresces and is coated with a white powder.

Identification A solution of Sodium Borate (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for borate.

pH <*2.54*> Dissolve 1.0 g of Sodium Borate in 20 mL of water: the pH of this solution is between 9.1 and 9.6.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Borate in 20 mL of water by warming slightly: the solution is clear and colorless.

(2) Carbonate or bicarbonate—Dissolve 1.0 g of powdered Sodium Borate in 20 mL of freshly boiled and cooled water, and add 3 mL of dilute hydrochloric acid: the solution does not effervesce.

(3) Heavy metals <*1.07*>—Dissolve 1.5 g of Sodium Borate in 25 mL of water and 7 mL of 1 mol/L hydrochloric acid TS, add 1 drop of phenolphthalein TS, and add ammonia TS until a pale red color develops. Then add dilute acetic acid until the solution becomes colorless again, add 2 mL of dilute acetic 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 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Sodium Borate according to Method 1, and perform the test (not more than 5 ppm).

Assay Weigh accurately about 2 g of Sodium Borate, dissolve in 50 mL of water, and titrate <*2.50*> with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.5 mol/L hydrochloric acid VS
= 95.34 mg of $Na_2B_4O_7.10H_2O$

Containers and storage Containers—Tight containers.

Sodium Bromide

臭化ナトリウム

NaBr: 102.89

Sodium Bromide, when dried, contains not less than 99.0% of NaBr.

Description Sodium Bromide occurs as colorless or white crystals or crystalline powder. It is odorless.

It is freely soluble in water, and soluble in ethanol (95).

It is hygroscopic, but not deliquescent.

Identification A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt and for bromide.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Bromide in 10 mL of water, add 0.10 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boil, and cool: the solution is colorless.

(3) Chloride—Make a calculation from the result obtained in the Assay. Not more than 97.9 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Sodium Bromide.

(4) Sulfate <*1.14*>—Perform the test with 2.0 g of Sodium Bromide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Sodium Bromide in 10 mL of freshly boiled and cooled water, and add 2 drops of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Bromide 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).

(8) Barium—Dissolve 0.5 g of Sodium Bromide in 10 mL

of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Sodium Bromide according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 5.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.4 g of Sodium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and 50 mL of 0.1 mol/L silver nitrate VS, exactly measured, and titrate <*2.50*> 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.

Each mL of 0.1 mol/L silver nitrate VS
= 10.29 mg of NaBr

Containers and storage Containers—Tight containers.

Sodium Carbonate Hydrate

炭酸ナトリウム水和物

$Na_2CO_3.10H_2O$: 286.14

Sodium Carbonate Hydrate contains not less than 99.0% and not more than 103.0% of $Na_2CO_3.10H_2O$.

Description Sodium Carbonate Hydrate occurs as colorless or white crystals.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Carbonate Hydrate (1 in 10) is alkaline.

It is efflorescent in air.

It liquefies in its water of crystallization at 34°C, and becomes anhydrous at above 100°C.

Identification A solution of Sodium Carbonate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for carbonate.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Carbonate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of Sodium Carbonate Hydrate in 10 mL of water, add 7 mL of dilute nitric acid, dilute with water to make 50 mL, and 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 0.071%).

(3) Heavy metals <*1.07*>—Dissolve 2.0 g of Sodium Carbonate Hydrate in 10 mL of water, add 8 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 8 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.65 of Sodium Carbonate Hydrate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying <*2.41*> 61.0～63.0% (1 g, 105°C, 4 hours).

Assay Dissolve about 3 g of Sodium Carbonate Hydrate, weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Boil cautiously, cool, and further titrate <*2.50*> until a greenish yellow color appears (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 143.1 mg of $Na_2CO_3.10H_2O$

Containers and storage Containers—Tight containers.

Dried Sodium Carbonate

乾燥炭酸ナトリウム

Na_2CO_3: 105.99

Dried Sodium Carbonate, when dried, contains not less than 99.0% of Na_2CO_3.

Description Dried Sodium Carbonate occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Dried Sodium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Dried Sodium Carbonate (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for carbonate.

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

(2) Chloride <*1.03*>—Dissolve 0.5 g of Dried Sodium Carbonate in 10 mL of water, add 12 mL of dilute nitric acid, dilute with water to make 50 mL, and 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 0.071%).

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water, add 7.5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7.5 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.65 g of Dried Sodium Carbonate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying <*2.41*> Not more than 2.0% (2 g, 106°C, 4 hours).

Assay Dissolve about 1.2 g of Dried Sodium Carbonate,

weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Then boil cautiously, cool, and further titrate <*2.50*> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 53.00 mg of Na_2CO_3

Containers and storage Containers—Tight containers.

Sodium Chloride

塩化ナトリウム

NaCl: 58.44

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Sodium Chloride contains not less than 99.0% and not more than 100.5% of NaCl, calculated on the dried basis.

◆**Description** Sodium Chloride occurs as colorless or white, crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

Identification **(1)** A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

◆**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Chloride in 5 mL of water: the solution is clear and colorless.◆

(2) Acidity or alkalinity—Dissolve 20.0 g of Sodium Chloride in 100.0 mL of freshly boiled and cooled water, and use this solution as the sample solution. To 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow. Separately, to 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is blue.

(3) Sulfates—To 7.5 mL of the sample solution obtained in (2) add water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted 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 minutes. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: any turbidity produced does not more than that produced in the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as directed above using this solution instead of the sample solution.

(4) Phosphates—To 2.0 mL of the sample solution obtained in (2) add 5 mL of 2 mol/L sulfuric acid TS and water to make 100.0 mL, then add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes: the color of the solution is not darker than the following control solution.

Control solution: To 1.0 mL of Standard Phosphoric Acid Solution add 12.5 mL of 2 mol/L sulfuric acid TS and water to make exactly 250 mL. To 100 mL of this solution add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes.

(5) Bromides—To 0.50 mL of the sample solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. After allowing to stand for 2 minutes, add 0.15 mL of 0.1 mol/L sodium thiosulfate VS, mix, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1,000,000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the control: the absorbance at 590 nm of the sample solution is not more than that of the standard solution.

(6) Iodides—Wet 5 g of Sodium Chloride with dropwisely added 0.15 mL of a freshly prepared mixture of starch TS, 0.5 mol/L sulfuric acid TS and sodium nitrite TS (1000:40:3), allow to stand for 5 minutes, and examine under daylight: a blue color does not appear.

(7) Ferrocyanides—Dissolve 2.0 g of Sodium Chloride in 6 mL of water, and add 0.5 mL of a mixture of a solution of iron (II) sulfate heptahydrate (1 in 100) and a solution of ammonium iron (III) sulfate dodecahydrate in diluted sulfuric acid (1 in 400) (1 in 100) (19:1): a blue color does not develop within 10 minutes.

◆**(8)** Heavy metals <*1.07*>—Proceed with 5.0 g of Sodium Chloride according to Method 1, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 3 ppm).◆

(9) Iron—To 10 mL of the sample solution obtained in (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, alkalize with ammonia TS, add water to make 20 mL, and allow to stand for 5 minutes: the solution has not more color than the following control solution.

Control solution: Pipet 1 mL of Standard Iron Solution, and add water to make exactly 25 mL. To 10 mL of this solution add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, and proceed in the same manner as directed for the sample solution.

(10) Barium—To 5.0 mL of the sample solution obtained in (2) add 5.0 mL of water and 2.0 mL of dilute sulfuric acid, and allow to stand for 2 hours: the solution has not more turbidity than the following control solution.

Control solution: To 5.0 mL of the sample solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.

(11) Magnesium and alkaline-earth materials—To 200 mL of water add 0.1 g of hydroxylammonium chloride, 10 mL of ammonium chloride buffer solution, pH 10, 1 mL of 0.1 mol/L zinc sulfate VS and 0.2 g of eriochrome black T-sodium chloride indicator, and warm to 40°C. Add 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS dropwise until the red-purple color of the solution changes to blue-purple. To this solution add a solution prepared by dissolving 10.0 g of Sodium Chloride in 100 mL of water, and add 2.5 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

◆**(12)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Sodium Chloride according to Method 1, and perform the test (not more than 2 ppm).◆

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, dissolve in 50 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

◆**Containers and storage** Containers—Tight containers.◆

10% Sodium Chloride Injection

10% 塩化ナトリウム注射液

10% Sodium Chloride Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	100 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description 10% Sodium Chloride Injection is a clear, colorless liquid. It has a saline taste.

It is neutral.

Identification 10% Sodium Chloride Injection responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt and for chloride.

Bactetial endotoxins ⟨*4.01*⟩ Less than 3.6 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 10 mL of 10% Sodium Chloride Injection, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate ⟨*2.50*⟩, with vigorous shaking, with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Isotonic Sodium Chloride Solution

0.9% Sodium Chloride Injection
Isotonic Salt Solution
Isotonic Sodium Chloride Injection

生理食塩液

Isotonic Sodium Chloride Solution is an aqueous solution for injection.

It contains not less than 0.85 w/v% and not more than 0.95 w/v% of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	9 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Isotonic Sodium Chloride Solution is a clear, colorless liquid. It has a slightly saline taste.

Identification Isotonic Sodium Chloride Solution responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt and for chloride.

pH ⟨*2.54*⟩ 4.5 – 8.0

Purity **(1)** Heavy metals ⟨*1.07*⟩—Concentrate 100 mL of Isotonic Sodium Chloride Solution to about 40 mL on a water bath, 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 3.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 20 mL of Isotonic Sodium Chloride Solution, and perform the test (not more than 0.1 ppm).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly 20 mL of Isotonic Sodium Chloride Solution, add 30 mL of water, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS with vigorous shaking (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Chromate (^{51}Cr) Injection

クロム酸ナトリウム (^{51}Cr) 注射液

Sodium Chromate (^{51}Cr) Injection is an aqueous solution for injection.

It contains a chromium-51 (^{51}Cr) in the form of sodium chromate.

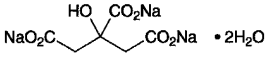
It conforms to the requirements of Sodium Chromate (^{51}Cr) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parentaral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Chromate (^{51}Cr) Injection is a clear, light yellow liquid. It is odorless or has an odor of the preservatives.

Sodium Citrate Hydrate

クエン酸ナトリウム水和物



$C_6H_5Na_3O_7.2H_2O$: 294.10
Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [*6132-04-3*]

Sodium Citrate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of sodium citrate ($C_6H_5Na_3O_7$: 258.07).

Description Sodium Citrate Hydrate occurs as colorless crystals, or a white, crystalline powder. It is odorless, and has a cooling, saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification A solution of Sodium Citrate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for citrate and for sodium salt.

pH <*2.54*> Dissolve 1.0 g of Sodium Citrate Hydrate in 20 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity **(1)** Clarity and color of solution—A solution of 1.0 g of Sodium Citrate Hydrate in 10 mL of water is clear and colorless.

(2) Chloride <*1.03*>—Take 0.6 g of Sodium Citrate Hydrate, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Sulfate <*1.14*>—To 0.5 g of Sodium Citrate Hydrate add water to make 40 mL, then add 3.0 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals <*1.07*>—Proceed with 2.5 g of Sodium Citrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Sodium Citrate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(6) Tartrate—To a solution of 1.0 g of Sodium Citrate Hydrate in 2 mL of water add 1 mL of potassium acetate TS and 1 mL of acetic acid (31): no crystalline precipitate is formed after the sides of the tube have been rubbed with a glass rod.

(7) Oxalate—Dissolve 1.0 g of Sodium Citrate Hydrate in a mixture of 1 mL of water and 3 mL of dilute hydrochloric acid, add 4 mL of ethanol (95) and 0.2 mL of calcium chloride TS, and allow to stand for 1 hour: the solution is clear.

(8) Readily carbonizable substances <*1.15*>—Take 0.5 g of Sodium Citrate Hydrate, and perform the test by heating at 90°C for 1 hour: the solution has no more color than Matching Fluid K.

Loss on drying <*2.41*> 10.0 – 13.0% (1 g, 180°C, 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Citrate Hydrate, previously dried, add 30 mL of acetic acid for nonaqueous titration, warm to dissolve, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.602 mg of $C_6H_5Na_3O_7$

Containers and storage Containers—Tight containers.

Sodium Citrate Injection for Transfusion

輸血用クエン酸ナトリウム注射液

Sodium Citrate Injection for Transfusion is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium citrate hydrate ($C_6H_5Na_3O_7.2H_2O$: 294.10).

Method of preparation

Sodium Citrate Hydrate	100 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservatives may be added.

Description Sodium Citrate Injection for Transfusion is a clear, colorless liquid.

Identification Sodium Citrate Injection for Transfusion responds to the Qualitative Tests <*1.09*> for sodium salt and for citrate.

pH <*2.54*> 7.0 – 8.5

Bacterial endotoxins <*4.01*> Less than 5.6 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 5 mL of Sodium Citrate Injection for Transfusion, and add water to make exactly 25 mL. Evaporate 10 mL of this solution, exactly measured, 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. Cool, titrate <*2.50*> with 0.1 mol/L perchloric acid VS (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
= 9.803 mg of $C_6H_5Na_3O_7.2H_2O$

Containers and storage Containers—Hermetic containers.

Diagnostic Sodium Citrate Solution

診断用クエン酸ナトリウム液

Diagnostic Sodium Citrate Solution contains not less than 3.3 w/v% and not more than 4.3 w/v% of sodium citrate hydrate ($C_6H_5Na_3O_7.2H_2O$: 294.10).

The requirements as described for aqueous injections under Injections are applicable.

Method of preparation

Sodium Citrate Hydrate	38 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Diagnostic Sodium Citrate Solution is a clear, colorless liquid.

Identification Diagnostic Sodium Citrate Solution responds to the Qualitative Tests <*1.09*> for sodium salt and for citrate.

pH <*2.54*> 7.0 – 8.5

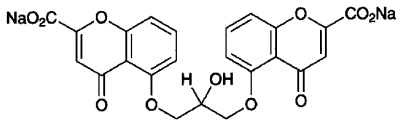
Assay Pipet 5 mL of Diagnostic Sodium Citrate Solution, evaporate on a water bath to dryness, dry the residue at 180°C for 2 hours, and dissolve in 30 mL of acetic acid (100) by warming. Cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.803 mg of $C_6H_5Na_3O_7.2H_2O$

Containers and storage Containers—Hermetic containers.

Sodium Cromoglicate

クロモグリク酸ナトリウム



$C_{23}H_{14}Na_2O_{11}$: 512.33
Disodium 5,5′-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4-oxo-4*H*-chromene-2-carboxylate)
[*15826-37-6*]

Sodium Cromoglicate contains not less than 98.0% of $C_{23}H_{14}Na_2O_{11}$, calculated on the dried basis.

Description Sodium Cromoglicate occurs as a white, crystalline powder. It is odorless and tasteless at first, and later develops a slightly bitter taste.

It is freely soluble in water, sparingly soluble in propylene glycol, very slightly soluble in ethanol (95), and practically insoluble in 2-propanol and in diethyl ether.

It is hygroscopic.

It gradually acquires a yellow color by light.

Identification **(1)** Dissolve 0.1 g of Sodium Cromoglicate in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute: a yellow color is produced. After cooling, add 0.5 mL of concentrated diazobenzene sulfonic acid TS: a dark red color is produced.

(2) Determine the absorption spectrum of a solution of Sodium Cromoglicate in phosphate buffer solution, pH 7.4 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Sodium Cromoglicate responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 0.50 g of Sodium Cromoglicate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Cromoglicate in 40 mL of freshly boiled and cooled water, add 6 drops of bromothymol blue TS, and use this solution as the sample solution. To 20 mL of the sample solution add 0.25 mL of 0.1 mol/L sodium hydroxide VS: a blue color is produced. To another 20 mL of the sample solution add 0.25 mL of 0.1 mol/L hydrochloric acid VS: a yellow color is produced.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Sodium Cromoglicate 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) Oxalate—Dissolve 0.25 g of Sodium Cromoglicate in water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 49 mg of oxalic acid dihydrate, exactly weighed, 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. Pipet 20 mL each of the sample solution and standard solution, add exactly 5 mL of iron salicylate TS to each solution, and add water to make 50 mL. Determine the absorbances of these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank: the absorbance of the sample solution at 480 nm is not smaller than that of the standard solution.

(5) Related substances—Dissolve 0.20 g of Sodium Cromoglicate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and acetic acid (100) (9:9:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 10.0% (1 g, in vacuum, 105°C, 4 hours).

Assay Weigh accurately about 0.18 g of Sodium Cromoglicate, and dissolve in a mixture of 25 mL of propylene glycol and 5 mL of 2-propanol by warming. After cooling, add 30 mL of 1,4-dioxane, and titrate <*2.50*> with 0.1 mol/L perchloric acid-1,4-ioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
= 25.62 mg of $C_{23}H_{14}Na_2O_{11}$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Disodium Edetate Hydrate

EDTA Sodium Hydrate

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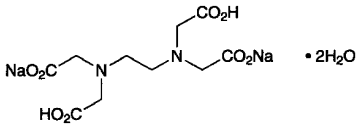

$C_{10}H_{14}N_2Na_2O_8.2H_2O$: 372.24
Disodium dihydrogen ethylenediaminetetraacetate dihydrate [*6381-92-6*]

Disodium Edetate Hydrate contains not less than 99.0% of $C_{10}H_{14}N_2Na_2O_8.2H_2O$.

Description Disodium Edetate Hydrate occurs as white crystals or crystalline powder. It is odorless and has a slight, acid taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification **(1)** Dissolve 0.01 g of Disodium Edetate Hydrate in 5 mL of water, add 2 mL of a solution of potassium chromate (1 in 200) and 2 mL of arsenic (III) trioxide TS, and heat in a water bath for 2 minutes: a purple color develops.

(2) Dissolve 0.5 g of Disodium Edetate Hydrate in 20 mL of water, and add 1 mL of dilute hydrochloric acid: a white precipitate is produced. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 1 hour: the precipitate melts <*2.60*> between 240°C and 244°C (with decomposition).

(3) A solution of Disodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> Dissolve 1 g of Disodium Edetate Hydrate in 100 mL of water: the pH of this solution is between 4.3 and 4.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Disodium Edetate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Cyanide—Transfer 1.0 g of Disodium Edetate Hydrate to a round-bottomed flask, dissolve in 100 mL of water, add 10 mL of phosphoric acid, and distil. Place 15 mL of 0.5 mol/L sodium hydroxide VS in a 100-mL measuring cylinder, which is used as a receiver, and immerse the bottom end of the condenser into the solution. Distil the mixture until the distillate measures 100 mL, and use this solution as the sample solution. Transfer 20 mL of the sample solution to a glass-stoppered test tube, add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, and add 5 mL of phosphate buffer solution, pH 6.8, and 1.0 mL of diluted sodium toluenesulfonchloramide TS (1 in 5). Immediately stopper the tube, mix gently, and allow to stand for a few minutes. Mix well with 5 mL of pyridine-pyrazolone TS, and allow to stand between 20°C and 30°C for 50 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, add 15 mL of 0.5 mol/L sodium hydroxide VS and water to make exactly 1000 mL, transfer 20 mL of this solution to a glass-stoppered test tube, and proceed as directed for the sample solution.

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Disodium Edetate 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 <*1.11*>—Prepare the test solution with 1.0 g of Disodium Edetate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Residue on ignition <*2.44*> 37.0 – 39.0% (1 g).

Assay Weigh accurately about 1 g of Disodium Edetate Hydrate, dissolve in 50 mL of water, add 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <*2.50*> with 0.1 mol/L zinc VS until the color of the solution changes from blue to red.

Each mL of 0.1 mol/L zinc VS
= 37.22 mg of $C_{10}H_{14}N_2Na_2O_8.2H_2O$

Containers and storage Containers—Well-closed containers.

Sodium Fusidate

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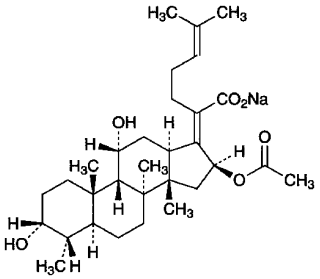


$C_{31}H_{47}NaO_6$: 538.69
Monosodium (17Z)-*ent*-16α-acetoxy-3β,11β-dihydroxy-4β,8β,14α-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oate
[*751-94-0*]

Sodium Fusidate is the sodium salt of a substance having antibacterial activity produced by the growth of *Fusidium coccineum*.

It contains not less than 935 μg (potency) and not more than 969 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sodium Fusidate is expressed as mass (potency) of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Description Sodium Fusidate occurs as white, crystals of crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectra of Sodium Fusidate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sodium Fusidate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Purity Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Fusidate 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).

Water <*2.48*> Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Diethanolamine Fusidate RS, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add 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 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 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 the low concentration standard solution, respectively.

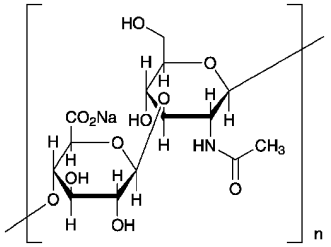
(iv) Sample solutions—Weigh accurately an amount of Sodium Fusidate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature 2 to 8°C.

Purified Sodium Hyaluronate

精製ヒアルロン酸ナトリウム



$(C_{14}H_{20}NNaO_{11})_n$
[*9067-32-7*]

Purified Sodium Hyaluronate is the sodium salt of glycosaminoglycans composed of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine obtained from cockscomb or microorganisms.

It contains not less than 90.0% and not more than 105.5% of sodium hyaluronate $(C_{14}H_{20}NNaO_{11})_n$, calculated on the dried basis.

It is composed of an average molecular mass of the sodium salt of hyaluronic acid between 500,000 and 1,200,000 or between 1,500,000 and 3,900,000.

The average molecular mass of Purified Sodium Hyaluronate should be labeled.

Description Purified Sodium Hyaluronate occurs as white powder, granules or fibrous masses.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Purified Sodium Hyaluronate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Purified Sodium Hyaluronate (1 in 1000) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Viscosity <*2.53*> Weigh accurately an amount of Purified Sodium Hyaluronate so that the downflowing time of its solution in 100 mL of 0.2 mol/L sodium chloride TS is 2.0 to 2.4 times longer than that of 0.2 mol/L sodium chloride TS, dissolve in 0.2 mol/L sodium chloride TS to make exactly 100 mL, and use this solution as the sample solution (1). Pipet 16 mL, 12 mL and 8 mL of the sample solution (1), to each add 0.2 mol/L sodium chloride TS to make exactly 20 mL, and use these solutions as the sample solutions (2), (3) and (4), respectively. Perform the test with the sample solutions (1), (2), (3) and (4) as directed under Method 1 at 30 ± 0.1°C using a Ubbelohde-type viscometer in which the downflowing time for 0.2 mol/L sodium chloride TS is 200 to 300 seconds: the intrinsic viscosity calculated on the dried basis is between 10.0 and 19.5 dL/g or between 25.0 and 55.0 dL/g.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Purified Sodium Hyaluronate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.20 g of Purified Sodium Hyaluronate in 15 mL of water, add 6 mL of dilute nitric acid, and heat on a water bath for 30 minutes. After cooling, 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 0.124%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Purified Sodium Hyaluronate 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) Residual solvent Being specified separately.

(5) Protein—Weigh accurately about 20 mg of Purified Sodium Hyaluronate, calculated on the dried basis, dissolve in 1.0 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of bovine serum albumin, dissolve in dilute sodium hydroxide TS to make exactly 1000 mL, and use this solution as the standard solution. To 1.0 mL each of the sample solution and standard solution add 5.0 mL of alkaline copper TS (2), immediately stir, allow to stand at room temperature for 10 minutes, add 0.5 mL of diluted Folin's TS (1 in 2), immediately stir, and allow to stand at room temperature for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 1.0 mL of dilute sodium hydrochloride in the same manner, as the blank: the absorbance of the sample solution at 750 nm does not exceed the absorbance of the standard solution (not more than 0.05%).

(6) Nucleic acid—Determine the absorbance of a solution of 0.10 g Purified Sodium Hyaluronate in 50 mL of water as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank: the absorbance at 260 nm is not more than 0.02.

(7) Other acidic mucopolysaccharides—(In the case of chicken-derived samples) Dissolve 0.25 g of Purified Sodium Hyaluronate in 100 mL of water, and use this solution as the sample solution. Immerse a cellulose acetate membrane 6 cm in length in 0.2 mol/L pirydine-formic acid buffer solution, pH 3.0. Take out the membrane and remove excessive buffer solution using a filter paper. Place the membrane in an electrophoresis vessel saturated with 0.2 mol/L pyridine-formic acid buffer solution, pH 3.0, and run at 0.5 mA/cm for 1 minute. Apply 2 μL of the sample solution to the membrane in an area 1 cm in width at 1.5 cm from the anode. Carry out electrophoresis at 0.5 mA/cm for 1 hour. After the electrophoresis, stain the membrane by immersing it in alcian blue staining solution for 10 to 20 minutes. After staining, decolorize sufficiently with diluted acetic acid (100) (3 in 100): no bands other than the principal band appears.

(8) Hemolytic streptococci—(In the case of microorganism-derived samples) Dissolve 0.5 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. Take 0.5 mL of this solution, apply to 2 blood agar plates, respectively, using a Conradi stick, and incubate at 37°C for 48 hours: no hemolytic colonies appear, or if any, no streptococci are observed in the colony under a microscope.

(9) Hemolysis—(In the case of microorganism-derived samples) Dissolve 0.40 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. To 0.5 mL of this solution add 0.5 mL of 1% blood suspension, mix, allow to stand at 37°C for 2 hours, and, if necessary, centrifuge at 3000 revolutions per minute for 10 minutes: the erythrocytes precipitate and the supernatant liquid is clear as in a blank determination performed in the same manner using 0.5 mL of sterile isotonic sodium chloride solution as the blank and 0.5 mL of sterile purified water as the positive control.

Loss on drying <*2.41*> Not more than 15.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 5 hours).

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 10^1 CFU/g.

Average molecular mass **(1)** In the case of the labeled average molecular mass of between 500,000 and 1,200,000.

Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 500,000 and 1,200,000. For $[\eta]$, use the maximum viscosity under Viscosity.

$$\text{Average molecular mass} = \left(\frac{[\eta] \times 10^5}{36}\right)^{\frac{1}{0.78}}$$

(2) In the case of the labeled average molecular mass of between 1,500,000 and 3,900,000.

Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 1,500,000 and 3,900,000. For $[\eta]$, use the maximum viscosity under Viscosity.

$$\text{Average molecular mass} = \left(\frac{[\eta] \times 10^5}{22.8}\right)^{\frac{1}{0.816}}$$

Assay Weigh accurately about 50 mg of Purified Sodium Hyaluronate, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of D-Glucuronolactone RS, previously dried (under reduced pressure not exceeding 0.67 kPa, silica gel, 24 hours), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make

exactly 10 mL, and use this solution as the standard solution. Pipet 1 mL each of the sample solution and standard solution, gently add into the 5.0 mL of sodium tetraborate-sulfuric acid TS, previously cooled in ice water, stir while cooling, heat in a water bath for 10 minutes, and cool in ice water. To each solution add exactly 0.2 mL of carbazole TS, stir well, heat in a water bath for 15 minutes, and cool in ice water to room temperature. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 530 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 1 mL of water in the same manner, as the blank.

Amount (mg) of sodium hyaluronate $[(C_{14}H_{20}NNaO_{11})_n]$ $= M_S \times A_T/A_S \times 2.279$

M_S: Amount (mg) of D-Glucuronolactone RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant, at not exceeding 15°C.

Sodium Hydroxide

水酸化ナトリウム

NaOH: 40.00

Sodium Hydroxide contains not less than 95.0% of NaOH.

Description Sodium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks, and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in moist air.

Identification **(1)** A solution of Sodium Hydroxide (1 in 500) is alkaline.

(2) A solution of Sodium Hydroxide (1 in 25) responds to the Qualitative Tests <1.09> for sodium salt.

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

(2) Chloride <1.03>—Dissolve 2.0 g of Sodium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 10 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 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Hydroxide in 5 mL of water, add 11 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Evaporate 11 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, add water to make 50 mL, and use this solution as the control solution (not more than 30 ppm).

(4) Potassium—Dissolve 0.10 g of Sodium Hydroxide in water and dilute with water to make 40 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, and shake. Add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and dilute with water to make 1000 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, shake, and proceed as directed above.

(5) Sodium carbonate—The amount of sodium carbonate (Na_2CO_3: 105.99) is not more than 2.0%, when calculated by the following equation using B (mL) which is obtained in the Assay.

Amount (mg) of sodium carbonate = $105.99 \times B$

(6) Mercury—Dissolve 2.0 g of Sodium Hydroxide in 1 mL of a solution of potassium permanganate (3 in 50) and 30 mL of water, neutralize gradually with purified hydrochloric acid, and add 5 mL of diluted sulfuric acid (1 in 2). To this solution add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 100 mL, and use this solution as the sample solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> (Cold vapor type) with the sample solution. Place the sample solution in the sample bottle of an atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Read the absorbance A_T of the sample solution when the indication of the recorder rises rapidly and becomes constant at the wavelength of 253.7 nm. On the other hand, to 2.0 mL of Standard Mercury Solution add 1 mL of a solution of potassium permanganate (3 in 50), 30 mL of water and a volume of purified hydrochloric acid equal to that used in the preparation of the sample solution, and read the absorbance A_S of the solution obtained by the same procedure as used for the sample solution: A_T is smaller than A_S.

Assay Weigh accurately about 1.5 g of Sodium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount, A (mL), of 0.5 mol/L sulfuric acid VS consumed. Then add 2 drops of methyl orange TS to the solution, and further titrate <2.50> with 0.5 mol/L sulfuric acid VS until the solution shows a persistent light red color. Record the amount, B (mL), of 0.5 mol/L sulfuric acid VS consumed. Calculate the amount of NaOH from the difference, A (mL) − B (mL).

Each mL of 0.5 mol/L sulfuric acid VS
= 40.00 mg of NaOH

Containers and storage Containers—Tight containers.

Sodium Iodide

ヨウ化ナトリウム

NaI: 149.89

Sodium Iodide, when dried, contains not less than 99.0% of NaI.

Description Sodium Iodide occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is very soluble in water, and freely soluble in glycerin and in ethanol (95).

It deliquesces in moist air.

Identification A solution of Sodium Iodide (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for iodide.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 1.0 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color is produced.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Sodium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for a few minutes, and filter. To 10 mL of the filtrate add 15 mL of dilute nitric acid: no brown color appears. The solution has no more turbidity than the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Sodium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert a pledget of absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on the cotton. Heat the test tube on a water bath for 15 minutes: the evolved gas does not turn moistened red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Sodium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, and add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Iodide 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).

(8) Barium—Dissolve 0.5 g of Sodium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Potassium—Dissolve 1.0 g of Sodium Iodide in water, and add water to make 100 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), immediately shake, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and add water to make 1000 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, and then proceed as directed above.

(10) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Sodium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 5.0% (2 g, 120°C, 2 hours).

Assay Weigh accurately about 0.4 g of Sodium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <*2.50*> with 0.05 mol/L potassium iodate VS while shaking vigorously until the red-purple color of the chloroform layer disappears. The end point is attained when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 14.99 mg of NaI

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sodium Iodide (^{123}I) Capsules

ヨウ化ナトリウム (^{123}I) カプセル

Sodium Iodide (^{123}I) Capsules contain iodine-123 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (^{123}I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (^{131}I) Capsules

ヨウ化ナトリウム (^{131}I) カプセル

Sodium Iodide (^{131}I) Capsules contain iodine-131 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (^{131}I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (^{131}I) Solution

ヨウ化ナトリウム (^{131}I) 液

Sodium Iodide (^{131}I) Solution contains iodine-131 (^{131}I) in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (^{131}I) Solution in the Minimum Requirements for Radiopharmaceuticals.

Description Sodium Iodide (^{131}I) Solution is a clear, colorless liquid. It is odorless, or has an odor due to the preservatives or stabilizers.

Sodium Iodohippurate (^{131}I) Injection

ヨウ化ヒプル酸ナトリウム (^{131}I) 注射液

Sodium Iodohippurate (^{131}I) Injection is an aqueous solution for injection containing iodine-131 (^{131}I) in the form of sodium *o*-iodohippurate.

It conforms to the requirements of Sodium Iodohippurate (^{131}I) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Iodohippurate (^{131}I) Injection is a clear, colorless liquid. It is odorless or has an odor of the preservatives or stabilizers.

Sodium Iotalamate Injection

イオタラム酸ナトリウム注射液

Sodium Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

Method of preparation

(1)		
Iotalamic Acid		645 g
Sodium Hydroxide		42 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity	
	To make	1000 mL
(2)		
Iotalamic Acid		772.5 g
Sodium Hydroxide		50.5 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity	
	To make	1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Sodium Iotalamate Injection is a clear, colorless or pale yellow, slightly viscous liquid.

It is gradually colored by light.

Identification **(1)** To a volume of Sodium Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate as directed in the Identification (2) under Iotalamic Acid.

(2) Sodium Iotalamate Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> 6.5 – 7.7

Purity **(1)** Primary aromatic amines—To a volume of Sodium Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—To a volume of Sodium Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, add 20 mL of water and 5 mL of dilute sulfuric acid, shake well, and filter the precipitate by suction through a glass filter (G4). To the filtrate add 5 mL of toluene, and shake vigorously: the toluene layer is colorless. Then add 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously: the toluene layer has no more color than the following control solution.

Control solution: Dissolve 0.25 g of potassium iodide in water to make 1000 mL. To 2.0 mL of this solution add 20 mL of water, 5 mL of dilute sulfuric acid, 5 mL of toluene and 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously.

Bacterial endotoxins <*4.01*> Less than 3.4 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Pipet a volume of Sodium Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of iotalamic acid to that of the internal standard.

$$\text{Amount (mg) of iotalamic acid } (C_{11}H_9I_3N_2O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of iotalamic acid for assay

Internal standard solution—A solution of L-tryptophan in the mobile phase (3 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside 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 20°C.

Mobile phase: To 3.9 g of phosphoric acid and 2.8 mL of triethylamine add water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of iotalamic acid 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, iotalamic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Sodium L-Lactate Solution

L-乳酸ナトリウム液

Sodium L-Lactate Solution is an aqueous solution of sodium salt of L-lactic acid.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium L-lactate ($C_3H_5NaO_3$).

The label states the content amount of sodium L-lactate.

Description Sodium L-Lactate Solution occurs as a clear and colorless viscous liquid. It has no odor or has a slight characteristic odor, and has a slight saline taste.

It is miscible with water or with ethanol (99.5).

Identification To an amount of Sodium L-Lactate Solution, equivalent to 1 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add water to make 50 mL. This solution responds to the Qualitative Tests <*1.09*> for sodium salt and for lactate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −38 – −44° To an exact amount of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 30 mL of water and 5.0 g of hexaammonium heptamolybdate tetrahydrate, then add water to make exactly 50 mL, and determine using a 100-mm cell.

pH <*2.54*> To an amount of Sodium L-Lactate Solution, equivalent to 5 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add water to make 50 mL: the pH of this solution is between 6.5 and 7.5.

Purity **(1)** Chloride <*1.03*>—Perform the test with an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate <*1.14*>—To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 7 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 0.010%).

(3) Heavy metals <*1.07*>—To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 5 mL of dilute hydrochloric acid, 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 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Iron <*1.10*>—Prepare the test solution with an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Arsenic <*1.11*>—To an amount of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, and add water to make 10 mL. Perform the test using 2 mL of this solution as the test solution (not more than 4 ppm).

(6) Sugars—To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 10 mL of water and 10 mL of Fehling's TS, and boil for 5 minutes: no red precipitate is produced.

(7) Citric, oxalic, phosphoric and L-tartaric acids—To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 1 mL of water and 1 mL of dilute hydrochloric acid, then add 40 mL of calcium hydroxide TS, and boil for 2 minutes: the solution is not changed.

(8) Volatile fatty acids—To an amount of Sodium L-Lactate Solution, equivalent to 3.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 2 mL of dilute sulfuric acid, and heat on a water bath: no acetic acid like nor lactic acid like odor is produced.

(9) Cyanide—Transfer an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, then add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color appears. Add further 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute hydrochloric acid until a red color of the solution disappears, then add 1 drop of acetic acid (31), 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. Add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the color of the solution is not more intense than that of the following control solution.

Control solution: To 1.0 mL of Standard Cyanide Solution add water to make 20 mL. Transfer 1.0 mL of this solu-

tion to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, then proceed in the same manner as descried above.

(10) Methanol—Transfer an amount of Sodium L-Lactate Solution, equivalent to 5.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, to a distilling flask of the apparatus for alcohol number determination <*1.01*>, add 10 mL of water, and distill. Pipet 5 mL of the distillate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to exactly 1.0 mL of methanol add water to make exactly 100 mL. Pipe 5 mL of this solution, add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions: the peak area of methanol obtained from the sample solution is not larger than that from the standard solution (not more than 0.025%).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography (149 – 177 μm in particle diameter).

Column temperature: A constant temperature of about 120°C.

Injection port and detector temperature: A constant temperature of about 125°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methanol is about 2 minutes.

System suitability—

System performance: To 1 mL of methanol and 1 mL of ethanol (99.5) add water to make 100 mL. To 5 mL of this solution add water to make 200 mL. To 5 mL of this solution add water to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methanol and ethanol 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanol is not more than 5%.

Assay Weigh accurately an amount of Sodium L-Lactate Solution, equivalent to about 0.25 g of sodium L-lactate ($C_3H_5NaO_3$), dry at 105°C for 4 hours, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of solution changes from purple to yellow-green through blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.21 mg of $C_3H_5NaO_3$

Containers and storage Containers—Tight containers.

Sodium Lauryl Sulfate

ラウリル硫酸ナトリウム

Sodium Lauryl Sulfate is a mixture of sodium alkyl sulfate consisting chiefly of sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$: 288.38).

Description Sodium Lauryl Sulfate occurs as white to light yellow crystals or powder. It has a slightly characteristic odor.

It is sparingly soluble in methanol and in ethanol (95).

A solution of Sodium Lauryl Sulfate (1 in 10) is a clear or an opalescent solution, which foams on agitation.

Identification **(1)** To 0.2 g of the residue obtained in Total alcohol content add 4 mL of bromine-cyclohexane TS with vigorous shaking, add 0.3 g of *N*-bromosuccinimide, and heat in a water bath at 80°C for 5 minutes: a red color develops.

(2) A solution of Sodium Lauryl Sulfate (1 in 10) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

(3) To a solution of Sodium Lauryl Sulfate (1 in 10) add dilute hydrochloric acid to make acid, boil gently, and cool: the solution responds to the Qualitative Tests <*1.09*> for sulfate.

Purity **(1)** Alkalinity—Dissolve 1.0 g of Sodium Lauryl Sulfate in 100 mL of water, add 2 drops of phenol red TS and 0.60 mL of 0.1 mol/L hydrochloric acid VS: the solution remains yellow.

(2) Sodium chloride—Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 50 mL of water, neutralize the solution with dilute nitric acid, if necessary, add exactly 5 mL of 0.1 mol/L sodium chloride TS, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (indicator: 2 drops of fluorescein sodium TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

The combined content of sodium chloride (NaCl: 58.44) and sodium sulfate (Na_2SO_4: 142.04) obtained in the next paragraph (3) is not more than 8.0%.

(3) Sodium sulfate—Dissolve about 1 g of Sodium Lauryl Sulfate, accurately weighed, in 10 mL of water, add 100 mL of ethanol (95), and heat at a temperature just below the boiling point for 2 hours. Filter through a glass filter (G4) while hot, and wash with 100 mL of boiling ethanol (95). Dissolve the precipitate by washing with 150 mL of water, collecting the washings in a beaker. Add 10 mL of hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, ignite to a constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of sodium sulfate (Na_2SO_4)
= amount (mg) of barium sulfate ($BaSO_4$) × 0.609

(4) Unsulfated alcohols—Dissolve about 10 g of Sodium Lauryl Sulfate, accurately weighed, in 100 mL of water, add

100 mL of ethanol (95), and transfer to a separator. Extract the solution with three 50-mL portions of petroleum benzin. If an emulsion forms, sodium chloride may be added to promote separation of the two layers. Combine the petroleum benzin extracts and wash with three 50-mL portions of water. Evaporate the petroleum benzin on a water bath, and dry the residue at 105°C for 30 minutes. The mass of the dried residue is not more than 4.0% of the mass of the Sodium Lauryl Sulfate.

Water ⟨*2.48*⟩ Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Total alcohol content Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 150 mL of water and 50 mL of hydrochloric acid, and boil under a reflux condenser for 4 hours. Cool, extract with two 75-mL portions of diethyl ether, and evaporate the combined diethyl ether extracts on a water bath. Dry the residue at 105°C for 30 minutes. The mass of the residue is not less than 59.0%.

Containers and storage Containers—Well-closed containers.

Sodium Pertechnetate (^{99m}Tc) Injection

過テクネチウム酸ナトリウム (^{99m}Tc) 注射液

Sodium Pertechnetate (^{99m}Tc) Injection is an aqueous solution for injection. It contains technetium-99*m* (^{99m}Tc) in the form of sodium pertechnetate.

It conforms to the requirements of Sodium Pertechnetate (^{99m}Tc) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Pertechnetate (^{99m}Tc) Injection is a clear, colorless liquid.

Dibasic Sodium Phosphate Hydrate

リン酸水素ナトリウム水和物

$Na_2HPO_4.12H_2O$: 358.14

Dibasic Sodium Phosphate Hydrate contains not less than 98.0% of disodium hydrogen phosphate (Na_2HPO_4: 141.96), calculated on the dried basis.

Description Dibasic Sodium Phosphate Hydrate occurs as colorless or white crystals. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It effloresces in warm, dry air.

Identification **(1)** A solution of Dibasic Sodium Phosphate Hydrate (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ (1) and (2) for sodium salt.

(2) A solution of Dibasic Sodium Phosphate Hydrate (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ (1) and (3) for phosphate.

(3) Dissolve 0.1 g of Dibasic Sodium Phosphate Hydrate in 5 mL of dilute nitric acid, warm at 70°C for 1 to 2 minutes, and add 2 mL of hexaammonium heptamolybdate TS: a yellow precipitate is formed.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 50 mL of water: the pH of this solution is between 9.0 and 9.4.

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

(2) Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 7 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 0.014%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.5 g of Dibasic Sodium Phosphate Hydrate in 2 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) Carbonate—To 2.0 g of Dibasic Sodium Phosphate Hydrate add 5 mL of water, boil, and add 2 mL of hydrochloric acid after cooling: the solution does not effervesce.

(5) Heavy metals ⟨*1.07*⟩—Dissolve 2.0 g of Dibasic Sodium Phosphate Hydrate in 4 mL of acetic acid (31) 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 10 ppm).

(6) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Dibasic Sodium Phosphate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying ⟨*2.41*⟩ 57.0 – 61.0% (1 g, at 40°C for 3 hours and then at 105°C for 5 hours, not exceeding 2 mm in sample layer).

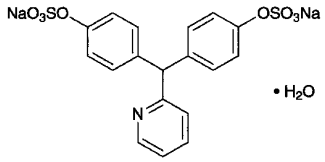
Assay Weigh accurately about 6 g of Dibasic Sodium Phosphate Hydrate, dissolve in 50 mL of water, and then titrate ⟨*2.50*⟩ with 0.5 mol/L sulfuric acid VS at 15°C until the green color of the solution changes to dark-greenish red-purple (indicator: 3 to 4 drops of methyl orange-xylenecyanol FF TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 142.0 mg of Na_2HPO_4

Containers and storage Containers—Tight containers.

Sodium Picosulfate Hydrate

ピコスルファートナトリウム水和物



$C_{18}H_{13}NNa_2O_8S_2.H_2O$: 499.42
Disodium 4,4′-(pyridin-2-ylmethylene)bis(phenyl sulfate) monohydrate
[*10040-45-6*, anhydride]

Sodium Picosulfate Hydrate contains not less than 98.5% of sodium picosulfate ($C_{18}H_{13}NNa_2O_8S_2$: 481.41), calculated on the anhydrous basis.

Description Sodium Picosulfate Hydrate occurs as a white, crystalline powder. It is odorless and tasteless.

It is very soluble in water, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is gradually colored by light.

The pH of a solution of Sodium Picosulfate Hydrate (1 in 20) is between 7.4 and 9.4.

Identification **(1)** Mix 5 mg of Sodium Picosulfate Hydrate with 0.01 g of 1-chloro-2,4-dinitrobenzene, and melt by gentle heating for 5 to 6 seconds. After cooling, add 4 mL of potassium hydroxide-ethanol TS: an orange-red color develops.

(2) To 0.2 g of Sodium Picosulfate Hydrate add 5 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and add 1 mL of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sodium Picosulfate Hydrate (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Sodium Picosulfate Hydrate, previously dried at 105° C in vacuum for 4 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Sodium Picosulfate Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (263 nm): 120 – 130 (calculated on the anhydrous basis, 4 mg, water, 100 mL).

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

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate <*1.14*>—Perform the test with 0.40 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.042%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Picosulfate 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) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Sodium Picosulfate Hydrate according to Method 3, and perform the test (not more than 1 ppm).

(6) Related substances—Dissolve 0.25 g of Sodium Picosulfate Hydrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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-butanol, water and acetic acid (100) (74:20:19) 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 sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 3.0 – 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.4 g of Sodium Picosulfate Hydrate, dissolve in 50 mL of methanol, add 7 mL of acetic acid (100), and titrate <*2.50*> 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
= 48.14 mg of $C_{18}H_{13}NNa_2O_8S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sodium Polystyrene Sulfonate

ポリスチレンスルホン酸ナトリウム

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.

It contains not less than 9.4% and not more than 11.0% of sodium (Na: 22.99), calculated on the anhydrous basis.

Each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, exchanges with not less than 0.110 g and not more than 0.135 g of potassium (K: 39.10).

Description Sodium Polystyrene Sulfonate occurs as a yellow-brown powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetone and in diethyl ether.

Identification **(1)** Determine the infrared absorption spectrum of Sodium Polystyrene Sulfonate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities

of absorption at the same wave numbers.

(2) To 1 g of Sodium Polystyrene Sulfonate add 10 mL of dilute hydrochloric acid, stir, and filter. Add ammonia TS to the filtrate to neutralize: the solution responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Ammonium—Place 1.0 g of Sodium 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.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium 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 <*1.11*>—Prepare the test solution with 2.0 g of Sodium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 1 ppm).

(4) Styrene—To 10.0 g of Sodium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. 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 exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine peak areas, A_T and A_S, of styrene in each solution: A_T is not larger than A_S.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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 water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of styrene is about 8 minutes.

System suitability—

System performance: Dissolve 20 mg each of styrene and butyl parahydroxybenzoate in 100 mL of acetone. To 5 mL of this solution add acetone to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and styrene 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of styrene is not more than 2.0%.

Water <*2.48*> Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Assay **(1)** Sodium—Weigh accurately about 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 50 mL of 3 mol/L hydrochloric acid TS, shake for 60 minutes, and filter. Discard the first 20 mL of the filtarte, pipet the subsequent 5 mL of the filtrate, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Sodium Stock Solution, dilute exactly with water so that each ml of the solution contains 1 to 3 μg of sodium (Na: 22.99), and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

(2) Potassium exchange capacity—Weigh accurately about 1.5 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 100 mL of Standard Potassium Stock Solution, shake for 15 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Potassium Stock Solution, dilute with water so that each mL of the solution contains an exact amount of 1 to 5 μg of potassium (K: 39.10), and use these solutions as the standard solutions. Perform the test with these solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount Y (mg) of potassium in 1000 mL of the sample solution using the calibration curve obtained from the standard solution. The quantity of potassium absorbed on each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated from the following equation: it is between 0.110 g and 0.135 g.

Quantity (mg) of potassium (K) absorbed on 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis
$= (X - 100Y)/M$

X: Amount (mg) of potassium in 100 mL of the Standard Potassium Stock Solution before exchange

M: Mass (g) of Sodium Polystyrene Sulfonate taken, calculated on the anhydrous basis

Gas: Combustible gas—Acetylene.
Supporting gas—Air.

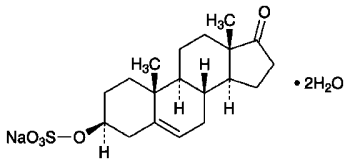
Lamp: A potassium hollow-cathode lamp.

Wavelength: 766.5 nm.

Containers and storage Containers—Tight containers.

Sodium Prasterone Sulfate Hydrate

プラステロン硫酸エステルナトリウム水和物



$C_{19}H_{27}NaO_5S.2H_2O$: 426.50
Monosodium 17-oxoandrost-5-en-3β-yl sulfate dihydrate
[*1099-87-2*, anhydride]

Sodium Prasterone Sulfate Hydrate contains not less than 98.0% of sodium prasterone sulfate ($C_{19}H_{27}NaO_5S$: 390.47), calculated on the dried basis.

Description Sodium Prasterone Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in acetone and in diethyl ether.

The pH of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) is between 4.5 and 6.5.

Melting point: about 160°C (with decomposition, after drying).

Identification **(1)** Dissolve 0.01 g of Sodium Prasterone Sulfate Hydrate in 4 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of a solution of sodium hydroxide (1 in 8): a red-purple color develops, and gradually changes to brown.

(2) To 10 mL of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) add 0.5 mL of bromine TS: the color of bromine TS immediately disappears.

(3) Determine the infrared absorption spectrum of Sodium Prasterone Sulfate Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Sodium Prasterone Sulfate Hydrate (1 in 200) responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +10.7 – +12.1° (0.73 g, calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g of Sodium Prasterone Sulfate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 1.0 g of Sodium Prasterone Sulfate Hydrate in 20 mL of acetone and 20 mL of water, 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 0.30 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(3) Sulfate <*1.14*>—To 1.2 g of Sodium Prasterone Sulfate Hydrate add 20 mL of water, shake vigorously for 5 minutes, and filter. To 10 mL of the filtrate add 20 mL of acetone, 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 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.032%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Sodium Prasterone Sulfate 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) Related substances—Dissolve 0.10 g of Sodium Prasterone Sulfate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 5 μL each of the sample 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, methanol and water (75:22:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> 8.0 – 9.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Weigh accurately about 0.25 g of Sodium Prasterone Sulfate Hydrate, dissolve in 30 mL of water. Apply this solution to a chromatographic column 10 mm in inside diameter, previously prepared by pouring 5 mL of strongly acidic ion-exchange resin (H type) for column chromatography, and elute at the rate of 4 mL per minute. Wash the chromatographic column with 100 mL of water, combine the washings with above effluent solution, and titrate <*2.50*> with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 19.52 mg of $C_{19}H_{27}NaO_5S$

Containers and storage Containers—Tight containers.

Sodium Pyrosulfite

Sodium Metabisulfite

ピロ亜硫酸ナトリウム

$Na_2S_2O_5$: 190.11

Sodium Pyrosulfite contains not less than 95.0% of $Na_2S_2O_5$.

Description Sodium Pyrosulfite occurs as white crystals or crystalline powder. It has the odor of sulfur dioxide.

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

A solution of Sodium Pyrosulfite (1 in 20) is acid.

It is hygroscopic.

It decomposes slowly on exposure to air.

Identification A solution of Sodium Pyrosulfite (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and for bisulfite.

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

(2) Thiosulfate—Dissolve 1.0 g of Sodium Pyrosulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water, and evaporate with 5 mL of hydrochloric acid on a water bath to dryness. Dissolve the residue in 10 mL of water, add 1 drop of phenolphthalein TS, and add ammonia TS until the solution becomes slightly red. 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: evaporate 5 mL of hydrochloric acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 1.0 g of Sodium Pyrosulfite according to Method 1, 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) Arsenic <*1.11*>—Dissolve 0.5 g of Sodium Pyrosulfite in 10 mL of water, heat with 1 mL of sulfuric acid on a sand bath until white fumes are evolved, and add water to make 5 mL. Perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Pyrosulfite, and transfer to an iodine flask containing an exactly measured 50 mL of 0.05 mol/L iodine VS. Stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate <*2.50*> the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

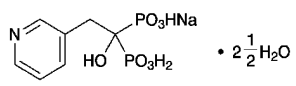
Each mL of 0.05 mol/L iodine VS = 4.753 mg of $Na_2S_2O_5$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Risedronate Hydrate

リセドロン酸ナトリウム水和物



$C_7H_{10}NNaO_7P_2.2\frac{1}{2}H_2O$: 350.13

Monosodium trihydrogen 1-hydroxy-2-(pyridin-3-yl)ethane-1,1-diyldiphosphonate hemipentahydrate

[*329003-65-8*]

Sodium Risedronate Hydrate contains not less than 98.0% and not more than 102.0% of sodium risedronate ($C_7H_{10}NNaO_7P_2$: 305.09), calculated on the anhydrous basis.

Description Sodium Risedronate Hydrate occurs as a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in diluted dilute sodium hydroxide TS (1 in 20).

Identification **(1)** Determine the absorption spectrum of a solution of Sodium Risedronate Hydrate in diluted dilute sodium hydroxide TS (1 in 20) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sodium Risedronate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Sodium Risedronate Hydrate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Purity **(1)** Heavy metals—To 0.50 g of Sodium Risedronate Hydrate in a quartz crucible add 0.50 g of magnesium oxide, mix, heat until the content becomes a light gray while mixing occasionally with a glass rod, then incinerate at 800°C. After cooling, dissolve the residue with 3 mL of hydrochloric acid, and add 3 mL of water. Adjust this solution to pH 8.5 with ammonia TS, then adjust to pH 4 with acetic acid (100), and adjust the pH to 3.4 with dilute hydrochloric acid. Filter the solution into a Nessler tube using a filter paper, rinse the crucible and filter with water, add the rinsings to the Nessler tube, then add water to make 50 mL, and use this solution as the test solution. Separately, to 1.0 mL of Standard Lead Solution add 0.50 g of magnesium oxide, dryness at 110°C, and proceed with the residue in the same manner as for the test solution, and use the solution so obtained as the control solution. To the test and control solutions add 1 drop each of sodium sulfate TS, mix, and allow to stand for 5 minutes, and compare the colors of both solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution by dissolving 1.0 g of Sodium Risedronate Hydrate in 5 mL of a solution of sodium hydroxide (1 in 5), and perform the test (not more than 2 ppm).

(3) Related substance 1—Dissolve 50 mg of Sodium Risedronate Hydrate in 1.5 mL of 0.2 mol/L sodium hydroxide TS, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 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 exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic acid 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.

Time span of measurement: About 2 times as long as the retention time of risedronic acid, beginning after the solvent peak.

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 risedronic acid are not less than 4500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 5.0%.

(4) Related substance 2—Dissolve 0.10 g of Sodium Risedronate Hydrate in 3 mL of 0.2 mL/L sodium hydroxide TS, add the diluting solution below to make 50 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the diluting solution to make exactly 50 mL. Pipet 2 mL of this solution, add the diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic acid from the standard solution.

Diluting solution: Dissolve 0.11 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 2.47 g of tetradecyl trimethylammonium bromide in 1000 mL of water, and adjust to pH 6.5 with 0.2 mL/L sodium hydroxide TS. To 700 mL of this solution add 300 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 0.14 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate, 3.16 g of tetradecyl trimethylammonium bromide, 4.81 g of ammonium dihydrogen phosphate and 2.93 g of diammonium hydrogen phosphate in 1280 mL of water, and add 720 mL of acetonitrile.

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

Time span of measurement: About 10 times as long as the retention time of risedronic acid, beginning after the solvent peak.

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 risedronic acid are not less than 5000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 2.0%.

(5) Residual solvent Being specified separately.

Water ⟨*2.48*⟩ 11.9 – 13.9% (40 mg, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (1:1) instead of methanol for Karl Fisher method).

Assay Weigh accurately about 50 mg of Sodium Risedronate Hydrate, dissolve in 1.5 mL of 0.2 mL/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water ⟨*2.48*⟩ using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of risedronic acid to that of the internal standard.

$$\text{Amount (mg) of sodium risedronate } (C_7H_{10}NNaO_7P_2) = M_S \times Q_T/Q_S \times 1.078$$

M_S: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A polyether ether ketone column 4 mm in inside diameter and 25 cm in length, packed with quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water, and adjust to pH 9.5 with 0.2 mL/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 14 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 risedronic acid 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Sodium Risedronate Tablets

リセドロン酸ナトリウム錠

Sodium Risedronate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium risedronate ($C_7H_{10}NNaO_7P_2$: 305.09).

Method of preparation Prepare as directed under Tablets, with Sodium Risedronate Hydrate.

Identification Powder Sodium Risedronate Tablets. To a portion of the powder, equivalent to 2.5 mg of sodium risedronate ($C_7H_{10}NNaO_7P_2$) according to the labeled amount, add 50 mL of diluted dilute sodium hydroxide TS (1 in 20), shake, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 2 mL of the filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 260 nm and 264 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Risedronate Tablets add exactly 10 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter the supenatant liquid through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 1 mL of the filtrate, pipet exactly V mL of the subsequent filtrate, equivalent to about 1.75 mg of sodium risedronate ($C_7H_{10}NNaO_7P_2$), add exactly 1 mL of the internal standard solution and the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Risedronic Acid RS (separately determine the water <*2.48*> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of risedronic acid to that of the internal standard.

Amount (mg) of sodium risedronate ($C_7H_{10}NNaO_7P_2$)
$= M_S \times Q_T/Q_S \times 1/V \times 1/4 \times 1.078$

M_S: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (7 in 2000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Sodium Risedronate Hydrate.

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 risedronic acid 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of risedronic acid to that of the internal standard is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Sodium Risedronate Tablets is not less than 80%.

Start the test with 1 tablet of Sodium Risedronate Tablets, withdraw 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of sodium risedronate ($C_7H_{10}NNaO_7P_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <*2.48*> using 80 mg in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add water to make exactly 50 mL. Pipet 5 mL of this solution, and add 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. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of risedronic acid of both solutions.

Dissolution rate (%) with respect to the labeled amount of sodium risedronate ($C_7H_{10}NNaO_7P_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2 \times 1.078$

M_S: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

C: Labeled amount (mg) of sodium risedronate ($C_7H_{10}NNaO_7P_2$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 0.15 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate, 3.36 g of tetradecyl trimethylammonium bromide, 5.11 g of ammonium dihydrogen phosphate and 3.11 g of diammonium hydrogen phosphate in 1360 mL of water, and add 640 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 12 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 5000

and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Sodium Risedronate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of sodium risedronate ($C_7H_{10}NNaO_7P_2$), add exactly 10 mL of the internal standard solution, add 190 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Sodium Risedronate Hydrate.

Amount (mg) of sodium risedronate ($C_7H_{10}NNaO_7P_2$)
$= M_S \times Q_T/Q_S \times 1.078$

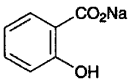
M_S: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (1 in 100).

Containers and storage Containers—Well-closed containers.

Sodium Salicylate

サリチル酸ナトリウム



$C_7H_5NaO_3$: 160.10
Monosodium 2-hydroxybenzoate
[*54-21-7*]

Sodium Salicylate, when dried, contains not less than 99.5% of $C_7H_5NaO_3$.

Description Sodium Salicylate occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and soluble in ethanol (95).

It is gradually colored by light.

Identification **(1)** Determine the infrared absorption spectrum of Sodium Salicylate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Sodium Salicylate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54> The pH of a solution of 2.0 g of Sodium Salicylate in 20 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Sodium Salicylate in 10 mL of water: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.02.

(2) Chloride <1.03>—Dissolve 0.5 g of Sodium Salicylate in 15 mL of water, add 6 mL of dilute nitric acid and ethanol (95) 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, 28 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate—Dissolve 0.25 g of Sodium Salicylate in 5 mL of water, and add 0.5 mL of barium chloride TS: the solution shows no change.

(4) Sulfite and thiosulfate—Dissolve 1.0 g of Sodium Salicylate in 20 mL of water, add 1 mL of hydrochloric acid, and filter. Add 0.15 mL of 0.05 mol/L iodine VS to the filtrate: a yellow color develops.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Sodium Salicylate 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 <1.11>—To 1.0 g of Sodium Salicylate in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. After cooling, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. Repeat the procedure of adding nitric acid and hydrogen peroxide (30) and heating, if necessary. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 0.3 g of Sodium Salicylate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> 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
$= 16.01$ mg of $C_7H_5NaO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sodium Starch Glycolate

デンプングリコール酸ナトリウム

[*9063-38-1*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Sodium Starch Glycolate is the sodium salt of a car-

boxymethyl ether of starch or of a cross-linked carboxymethyl ether of starch.

There are two neutralization types of Sodium Starch Glycolate, Type A and Type B, and their insoluble matter in a mixture of ethanol (99.5) and water (8:2), when dried, contains not less than 2.8% and not more than 4.2%, and not less than 2.0% and not more than 3.4% of sodium (Na: 22.99), respectively.

◆The label states the type of neutralization.◆

◆**Description** Sodium Starch Glycolate occurs as a white powder, and has a characteristic salty taste.

It practically insoluble in ethanol (99.5).

It swells with water, and becomes viscous, pasty liquid.

It is hygroscopic.◆

Identification **(1)** Acidify 5 mL of a solution of Sodium Starch Glycolate (1 in 500) with dilute hydrochloric acid, then add one drop of iodine TS, and stir: a blue to violet color is produced.

◆**(2)** Determine the infrared absorption spectrum of Sodium Starch Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

(3) The sample solution obtained in the Purity (2) responds to the Qualitative Tests <*1.09*> (2) for sodium salt. Perform the test using 2 mL of the sample solution and 4 mL of potassium hexahydroxoantimonate (V) TS.

pH <*2.54*> To 1 g of Sodium Starch Glycolate add 30 mL of water and stir: the pH of the resulting suspension of Type A is 5.5 – 7.5, and that of Type B is 3.0 – 5.0.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Sodium Starch Glycolate 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) Iron

(i) Sample solution Take 2.5 g of Sodium Starch Glycolate in a silica or platinum crucible, add 2 mL of 5 mol/L sulfuric acid TS. Heat on a water bath, then ignite cautiously with a gas burner or preferably in an electric furnace at 600 ± 25°C, and incinerate the residue completely. Allow to cool, add a few drops of 1 mol/L sulfuric acid TS, and heat and ignite as above. Allow to cool, add a few drops of ammonium carbonate TS, evaporate to dryness on a water bath, and heat and ignite as above. After cooling, dissolve the residue by adding 50 mL of water.

(ii) Standard solution Weigh accurately 863.4 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in water, add 25 mL of 1 mol/L sulfuric acid TS, and add water to make exactly 500 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 1.0 μg of iron (Fe).

(iii) Procedure Pipet 10 mL each of the sample solution and standard solution, and to each solution add 2 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid. Then add ammonia solution (28) dropwise to render the solution alkaline, using litmus paper as an indicator. Add water to make 20 mL, and use these solutions as the test solution and the control solution, respectively. Allow these solutions to stand for 5 minutes, and compare the color of the solutions using white background: the color of the test solution is not deeper than that of the control solution (not more than 20 ppm).

(3) Sodium glycolate—Conduct this procedure without exposure to light, using light-resistant vessels.

(i) Sample solution Weigh accurately 0.200 g of Sodium Starch Glycolate in a beaker, add 4 mL of 6 mol/L acetic acid TS and 5 mL of water, and stir to dissolve. Add 50 mL of acetone and 1 g of sodium chloride, stir, and filter through a filter paper previously soaked with acetone. Rinse the beaker and the filter paper with acetone, combine the filtrate and washings, and add acetone to make exactly 100 mL. Allow to stand for 24 hours, and use the supernatant liquid as the sample solution.

(ii) Standard solution To exactly 0.310 g of glycolic acid, previously dried in a desiccator (silica gel) for 18 hours, add water to dissolve to make exactly 500 mL. Pipet 5 mL of this solution, add 4 mL of 6 mol/L acetic acid TS, and allow to stand for 30 minutes. Add 50 mL of acetone and 1 g of sodium chloride, proceed as (i) above, and use the supernatant liquid as the standard solution.

(iii) Procedure Pipet 2.0 mL each of the sample solution and standard solution into 25-mL stoppered test tubes, and heat on a water bath for 20 minutes to remove acetone. After cooling, add 20.0 mL of 2,7-dihydroxynaphthalene TS to the residue, stopper the test tube, and heat on a water bath for 20 minutes. Cool under running water, and transfer whole quantity of the content to a 25-mL volumetric flask. Maintain the flask under running water, and add sulfuric acid to make 25 mL. Within 10 minutes, determine the absorbance of these solutions at 540 nm using water as the blank as directed under Ultraviolet-visible Spectrophotometry <*2.24*>; the absorbance of the sample solution is not larger than that of the standard solution (not more than 2.0%).

(4) Sodium chloride—Weigh accurately about 0.5 g of Sodium Starch Glycolate in a beaker, disperse in 100 mL of water, and add 1 ml of nitric acid. Titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration): the amount of sodium chloride (NaCl: 58.44) is not more than 7.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

Loss on drying <*2.41*> Not more than 10.0% (1 g, 130°C, 90 minutes).

Assay To about 1 g of Sodium Starch Glycolate add 20 mL of a mixture of ethanol (99.5) and water (8:2), stir for 10 minutes, and filter. Repeat this procedure until no more turbidity is produced by adding silver nitrate TS, and dry the residue on the filter paper at 105°C to constant mass. Weigh accurately 0.7 g of the mass, add 80 mL of acetic acid (100), and heat the mixture under a reflux condenser on a water bath for 2 hours. After cooling, titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration).

$$\text{Content (\%) of sodium (Na)} = (V \times 2.299 \times 100)/M$$

V: Consumed amount (mL) of 0.1 mol/L perchloric acid VS

M: Mass (mg) of the dried residue

◆**Containers and storage** Containers—Tight containers.◆

Dried Sodium Sulfite

乾燥亜硫酸ナトリウム

Na_2SO_3: 126.04

Dried Sodium Sulfite contains not less than 97.0% of Na_2SO_3.

Description Dried Sodium Sulfite is white crystals or powder. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Dried Sodium Sulfite (1 in 10) is about 10.

It gradually changes in moist air.

Identification An aqueous solution of Dried Sodium Sulfite (1 in 20) responds to the Qualitative Tests <*1.09*> for sodium salt and sulfite.

Purity **(1)** Thiosulfate—Dissolve 1.0 g of Dried Sodium Sulfite in 15 mL of water, add gradually 5 mL of hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(2) Heavy metals <*1.07*>—Dissolve 1.0 g of Dried Sodium Sulfite in 5 mL of water, add 2 mL of hydrochloric acid gradually, and evaporate the mixture on a water bath to dryness. Add 3 mL of boiling water and 1 mL of hydrochloric acid to the residue, and again evaporate to dryness on a water bath. Dissolve the residue in 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: evaporate 3 mL of hydrochloric acid to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Arsenic <*1.11*>—Dissolve 0.5 g of Dried Sodium Sulfite in 5 mL of water, add 1 mL of sulfuric acid, and evaporate on a sand bath until white fumes are evolved. Add water to make 5 mL, take this solution as the sample solution, and perform the test (not more than 4 ppm).

Assay Weigh accurately about 0.2 g of Dried Sodium Sulfite, transfer immediately to an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate <*2.50*> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 6.302 mg of Na_2SO_3

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Hydrate

チオ硫酸ナトリウム水和物

$Na_2S_2O_3.5H_2O$: 248.18

Sodium Thiosulfate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of sodium thiosulfate ($Na_2S_2O_3$: 158.11).

Description Sodium Thiosulfate Hydrate occurs as colorless, crystals or crystalline powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It effloresces in dry air, and is deliquescent in moist air.

Identification **(1)** A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for thiosulfate.

(2) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

pH <*2.54*> Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

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

(2) Heavy metals <*1.07*>—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add slowly 5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Add 15 mL of water to the residue, boil gently for 2 minutes, and filter. Heat the filtrate to boil, and add bromine TS to the hot filtrate to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the bromine. Cool, add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until a slight red color is produced. 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 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Calcium—Dissolve 1.0 g of Sodium Thiosulfate in 10 mL of water, add 2 mL of ammonium oxalate TS, and allow to stand for 4 minutes: no turbidity is produced.

(4) Arsenic <*1.11*>—To 0.40 g of Sodium Thiosulfate add 3 mL of nitric acid and 5 mL of water, evaporate on a water bath to dryness, and perform the test with the residue. Prepare the test solution according to Method 2, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> 32.0 – 37.0% (1 g, in vacuum, 40 – 45°C, 16 hours).

Assay Weigh accurately about 0.4 g of Sodium Thiosulfate, previously dried, dissolve in 30 mL of water, and titrate <*2.50*> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 15.81 mg of $Na_2S_2O_3$

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Injection

チオ硫酸ナトリウム注射液

Sodium Thiosulfate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium thiosulfate hydrate ($Na_2S_2O_3.5H_2O$: 248.18).

Method of preparation Prepare as directed under Injections, with Sodium Thiosulfate Hydrate.

Description Sodium Thiosulfate Injection is a clear, colorless liquid.

Identification Sodium Thiosulfate Injection responds to the Qualitative Tests <*1.09*> for sodium salt and for thiosulfate.

Bacterial endotoxins <*4.01*> Less than 0.01 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

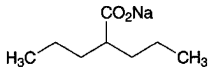
Assay Measure exactly a volume of Sodium Thiosulfate Injection, equivalent to about 0.5 g of sodium thiosulfate hydrate ($Na_2S_2O_3.5H_2O$), add water to make 30 mL, and titrate <*2.50*> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 24.82 mg of $Na_2S_2O_3.5H_2O$

Containers and storage Containers—Hermetic containers.

Sodium Valproate

バルプロ酸ナトリウム



$C_8H_{15}NaO_2$: 166.19
Monosodium 2-propylpentanoate
[*1069-66-5*]

Sodium Valproate, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{15}NaO_2$.

Description Sodium Valproate occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5) and in acetic acid (100).

It is hygroscopic.

Identification **(1)** To 5 mL of a solution of Sodium Valproate (1 in 20) add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

(2) Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of diethyl ether and 1 mL of 2 mol/L hydrochloric acid TS, and shake vigorously for 1 minute. Separate the diethyl ether layer, dehydrate with anhydrous sodium sulfate, and filter. Evaporate the solvent of the filtrate, determine the infrared spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Sodium Valproate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

pH <*2.54*> Dissolve 1.0 g of Sodium Valproate in 20 mL of water: the pH of this solution is between 7.0 and 8.5.

Purity **(1)** Heavy metals <*1.07*>—Dissolve 2.0 g of Sodium Valproate in 44 mL of water, shake with 6 mL of dilute hydrochloric acid, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent filtrate 25 mL 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 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).

(2) Related substances—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and methyl acetate (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of formic acid and methyl acetate (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of valproic acid from the sample solution is not larger than the peak area of valproic acid from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with diethylene glycol adipate ester for gas chromatography and phosphoric acid at the ratios of 5% and 1%, respectively.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of valproic acid is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of valproic acid, beginning after the solvent peak.

System suitability—

System performance: To 2 mL of the sample solution and 8 μL of *n*-valerianic acid, add a mixture of formic acid and methyl acetate (1:1) to make 10 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, *n*-valerianic acid and valproic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: Pipet 2 mL of the standard solution and add a mixture of formic acid and methyl acetate (1:1) to make exactly 10 mL. When the test is repeated 6 times with 2 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 5.0%.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Sodium Valproate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potenti-

ometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.62 mg of $C_8H_{15}NaO_2$

Containers and storage Containers—Tight containers.

Sodium Valproate Syrup

バルプロ酸ナトリウムシロップ

Sodium Valproate Syrup contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Syrups, with Sodium Valproate.

Identification To a volume of Sodium Valproate Syrup, equivalent to 50 mg of Sodium Valproate according to the labeled amount, add water to make 10 mL. To 5 mL of this solution add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^1 CFU/mL, respectively. *Escherichia coli* is not observed.

Assay Pipet a volume of Sodium Valproate Syrup, equivalent to about 0.1 g of sodium valproate ($C_8H_{15}NaO_2$) and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sodium valproate for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 50 mL. Pipet 20 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of valproic acid to that of the internal standard.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of valproic acid 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, the internal standard and valproic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sodium Valproate Tablets

バルプロ酸ナトリウム錠

Sodium Valproate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Tablets, with Sodium Valproate.

Identification To a quantity of powdered Sodium Valproate Tablets, equivalent to 0.5 g of Sodium Valproate according to the labeled amount, add 10 mL of water, shake well, and centrifuge. To 5 mL of the supernatant liquid add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Valproate Tablets add $7V/10$ mL of the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 1 mg of sodium valproate ($C_8H_{15}NaO_2$), and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, shake vigorously, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sodium Valproate Tablets is not less than 85%.

Start the test with 1 tablet of Sodium Valproate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.11 mg of sodium valproate ($C_8H_{15}NaO_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of sodium valproate for assay, previously dried at 105°C for

3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL of the solution, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of the valproic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of sodium valproate ($C_8H_{15}NaO_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of sodium valproate for assay
C: Labeled amount (mg) of sodium valproate ($C_8H_{15}NaO_2$) 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of valproic acid are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Sodium Valproate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of sodium valproate ($C_8H_{15}NaO_2$), add about 160 mL of the mobile phase, shake well, add the mobile phase to make exactly 200 mL, and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of sodium valproate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 20 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of valproic acid to that of the internal standard.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside 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 0.05 mol/L sodium dihydrogen phosphate TS, pH 3.0 and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of valproic acid 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, the internal standard and valproic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sorbitan Sesquioleate

ソルビタンセスキオレイン酸エステル

Sorbitan Sesquioleate is a mixture of monoester and diester of sorbitol anhydride, partially esterified with oleic acid.

Description Sorbitan Sesquioleate is a pale yellow to light yellow-brown, viscous oily liquid. It has a faint, characteristic odor and a slightly bitter taste.
It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and very slightly soluble in methanol.
It is dispersed as fine oily drops in water.

Identification **(1)** To 0.5 g of Sorbitan Sesquioleate add 5 mL of ethanol (95) and 5 mL of dilute sulfuric acid, and heat on a water bath for 30 minutes. Cool, shake with 5 mL of petroleum ether, and allow to stand, and separate the upper layer and the lower layer. Shake 2 mL of the lower layer with 2 mL of freshly prepared catechol solution (1 in 10), then with 5 mL of sulfuric acid: a red to red-brown color develops.
(2) Heat the upper layer obtained in (1) on a water bath, and evaporate petroleum ether. To the residue add 2 mL of diluted nitric acid (1 in 2), and then add 0.5 g of potassium nitrite between 30°C and 35°C with stirring: the solution develops an opalescence, and, when cooled, crystals are formed.

Specific gravity <*1.13*> d_{25}^{25}: 0.960 – 1.020

Saponification value <*1.13*> 150 – 168

Purity **(1)** Acidity—To 2.0 g of Sorbitan Sesquioleate add 50 mL of neutralized ethanol, and heat on a water bath nearly to boiling with stirring once or twice. Cool, add 4.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.
(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Sorbitan Sesquioleate 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 <*1.11*>—Prepare the test solution with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 3.0% (1 g, volumetric titra-

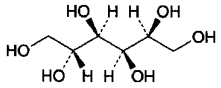
tion, direct titration, stir for 30 minutes).

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (1 g).

Containers and storage Containers—Tight containers.

D-Sorbitol

D-ソルビトール



$C_6H_{14}O_6$: 182.17
D-Glucitol
[*50-70-4*]

D-Sorbitol, when dried, contains not less than 97.0% of $C_6H_{14}O_6$.

Description D-Sorbitol occurs as white granules, powder, or crystalline masses. It is odorless, and has a sweet taste with a cold sensation.

It is very soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** To 1 mL of a solution of D-Sorbitol (7 in 10) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

(2) Shake thoroughly 1 mL of a solution of D-Sorbitol (1 in 20) with 1 mL of a freshly prepared solution of catechol (1 in 10), add rapidly 2 mL of sulfuric acid, and shake: a reddish purple to red-purple color immediately develops.

(3) Boil 0.5 g of D-Sorbitol with 10 mL of acetic anhydride and 1 mL of pyridine under a reflux condenser for 10 minutes, cool, shake with 25 mL of water, and allow to stand in a cold place. Transfer the solution to a separator, extract with 30 mL of chloroform, and evaporate the extract on a water bath. Add 80 mL of water to the oily residue, heat for 10 minutes on a water bath, then filter the hot mixture. After cooling, collect the produced precipitate through a glass filter (G3), wash with water, recrystallize once from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: the precipitate melts between 97°C and 101°C.

Purity **(1)** Clarity and color of solution, and acidity or alkalinity—Dissolve 5 g of D-Sorbitol in 20 mL of water by warming with shaking: the solution is clear, colorless, and neutral.

(2) Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of D-Sorbitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 4.0 g of D-Sorbitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of D-Sorbitol 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).

(5) Nickel—Dissolve 0.5 g of D-Sorbitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(6) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.5 g of D-Sorbitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Glucose—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and boil gently with 40 mL of Fehling's TS for 3 minutes. After cooling, filter the supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show an alkali reaction, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash with water. Combine the filtrate and the washings, heat at 80°C, and titrate ⟨*2.50*⟩ with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of volume for titration consumed or consumption is required.

(8) Sugars—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and heat with 8 mL of dilute hydrochloric acid under a reflux condenser in a water bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops, and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.02% (5 g).

Assay Weigh accurately about 0.2 g of D-Sorbitol, previously dried, dissolve in water and add water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Tight containers.

D-Sorbitol Solution

D-ソルビトール液

D-Sorbitol Solution contains not less than 97.0% and not more than 103.0% of the labeled amount of D-sorbitol ($C_6H_{14}O_6$: 182.17).

Description D-Sorbitol Solution is a clear, colorless liquid. It is odorless, and has a sweet taste.

It is miscible with water, with ethanol (95), with glycerin and with propylene glycol.

It sometimes separates crystalline masses.

Identification **(1)** To a volume of D-Sorbitol Solution, equivalent to 0.7 g of D-Sorbitol according to the labeled amount, add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

(2) To a volume of D-Sorbitol Solution, equivalent to 1 g of D-Sorbitol according to the labeled amount, add water to make 20 mL. To 1 mL of this solution add 1 mL of a freshly prepared solution of catechol (1 in 10), mix well, then add rapidly 2 mL of sulfuric acid, and mix: a reddish purple to red-purple color immediately develops.

Purity **(1)** Acidity or alkalinity—D-Sorbitol Solution is neutral.

(2) Chloride <*1.03*>—Proceed with a volume of D-Sorbitol Solution, equivalent to 2.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <*1.14*>—To a volume of D-Sorbitol Solution, equivalent to 4.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <*1.07*>—Proceed with a volume of D-Sorbitol Solution, equivalent to 5.0 g of D-Sorbitol according to the labeled amount, and according to Method 1, perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Take a volume of D-Sorbitol Solution, equivalent to 0.5 g of D-Sorbitol according to the labeled amount, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(6) Arsenic <*1.11*>—Take a volume of D-Sorbitol Solution, equivalent to 1.5 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 5 mL on a water bath, if necessary, cool, and perform the test using this solution as the test solution (not more than 1.3 ppm).

(7) Glucose—Take a volume of D-Sorbitol Solution, equivalent to 20.0 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 40 mL on a water bath, if necessary, add 40 mL of Fehling's TS, and boil gently for 3 minutes. After cooling, filter the supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show alkalinity, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash the filter with water. Combine the filtrate and the washings, heat at 80°C, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of 0.02 mol/L potassium permanganate VS is required.

(8) Sugars—Take a volume of D-Sorbitol Solution, equivalent to 20.0 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 40 mL of a water bath, if necessary, add 8 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops, and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Residue on ignition <*2.44*> Measure exactly a volume of D-Sorbitol Solution, equivalent to 5 g of D-Sorbitol according to the labeled amount, add 3 to 4 drops of sulfuric acid, and heat gently to evaporate. Ignite to burn, cool, and perform the test with the residue: not more than 1 mg.

Assay Measure exactly a volume of D-Sorbitol Solution, equivalent to about 5 g of D-sorbitol ($C_6H_{14}O_6$) according to the labeled amount, and add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Tight containers.

Soybean Oil

Oleum Sojae

ダイズ油

Soybean Oil is the fixed oil obtained from the seeds of *Glycine max* Merrill (*Leguminosae*).

Description Soybean Oil is a clear, pale yellow oil. It is odorless or has a slight odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It congeals between −10°C and −17°C.

Congealing point of the fatty acids: 22 – 27°C

Specific gravity <*1.13*> d_{25}^{25}: 0.916 – 0.922

Acid value <*1.13*> Not more than 0.2.

Saponification value <*1.13*> 188 – 195

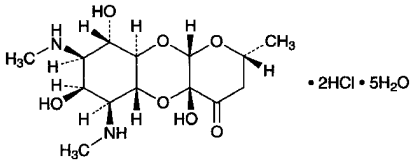
Unsaponifiable matter <*1.13*> Not more than 1.0%.

Iodine value <*1.13*> 126 – 140

Containers and storage Containers—Tight containers.

Spectinomycin Hydrochloride Hydrate

スペクチノマイシン塩酸塩水和物



$C_{14}H_{24}N_2O_7.2HCl.5H_2O$: 495.35

(2*R*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-4a,7,9-Trihydroxy-2-methyl-6,8-bis(methylamino)-2,3,4a,5a,6,7,8,9,9a,10a-decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate

[*22189-32-8*]

Spectinomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces spectabilis*.

It contains not less than 603 μg (potency) and not more than 713 μg (potency) per mg. The potency of Spectinomycin Hydrochloride Hydrate is expressed as mass (potency) of spectinomycin ($C_{14}H_{24}N_2O_7$: 332.35).

Description Spectinomycin Hydrochloride Hydrate occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** To 5 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 100) add gently anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared absorption spectra of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride RS as directed in the paste method under the Infrared Spectrophotometry <*2.25*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 150) add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +15 – +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm).

pH <*2.54*> Dissolve 0.10 g of Spectinomycin Hydrochloride Hydrate in 10 mL of water: the pH of the solution is between 4.0 and 5.6.

Water <*2.48*> Not less than 16.0% and not more than 20.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Klebsiella pneumoniae* ATCC 10031

(ii) Culture medium—Use the medium i in 3) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

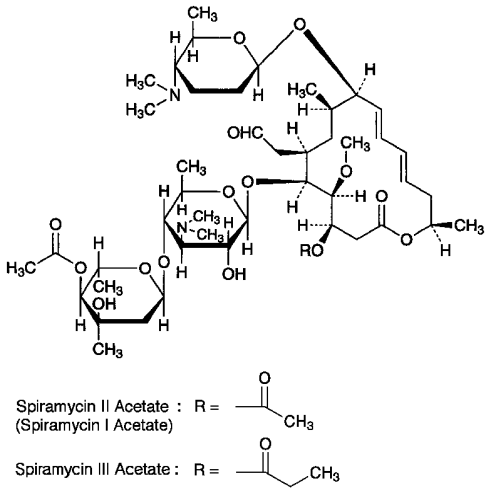
(iii) Standard solutions—Weigh accurately an amount of Spectinomycin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. 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 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Hydrate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 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 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spiramycin Acetate

スピラマイシン酢酸エステル



(Spiramycin II Acetate (Spiramycin I Acetate))
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-9-(2,3,4,6-tetradeoxy-4-dimethylamino-β-D-*erythro*-hexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide
(Spiramycin III Acetate)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acetyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-9-(2,3,4,6-tetradeoxy-4-dimethylamino-β-D-*erythro*-hexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide
[*74014-51-0*, Spiramycin Acetate]

Spiramycin Acetate is a derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambofaciens*.

It contains not less than 900 μg (potency) and not more than 1450 μg (potency) per mg, calculated on the dried basis. The potency of Spiramycin Acetate is expressed as mass (potency) of spiramycin II acetate ($C_{47}H_{78}N_2O_{16}$: 927.13). One mg (potency) of Spiramycin Acetate is equivalent to 0.7225 mg of spiramycin II acetate ($C_{47}H_{78}N_2O_{16}$).

Description Spiramycin Acetate occurs as a white to light yellowish white powder.

It is very soluble in acetonitrile and in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Spiramycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spiramycin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Content ratio of the active principle Dissolve 25 mg of Spiramycin Acetate in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas, A_{II}, A_{III}, A_{IV}, A_V, A_{VI} and A_{VII}, of the peaks of spiramycin II acetate, spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate, respectively, by the automatic integration method, and calculate the ratios of the amounts of A_{II}, A_{IV} and the total of A_{III} and A_V to the total amount of all these peaks: the amount of A_{II} is 30 – 45%, A_{IV} is 30 – 45%, and the total of A_{III} and A_V is not more than 25%. The relative retention times of spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate with respect to spiramycin II acetate are 1.3, 1.7, 2.3, 0.85 and 1.4, respectively.

Operating conditions—

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

Column: A stainless steel column 6 mm in inside diameter and 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 25,000) (26:7:7).

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

System suitability—

System performance: Dissolve 25 mg of Spiramycin II Acetate 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 the symmetry factor of the peak of spiramycin II acetate are not less than 14,500 and not more than 2.0, respectively.

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

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Spiramycin Acetate 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 <*1.11*>—Prepare the test solution with 2.0 g of Spiramycin Acetate according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying <*2.41*> Not more than 3.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

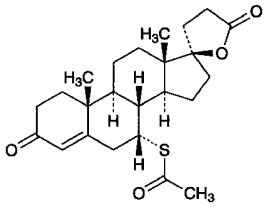
(iii) Standard solutions—Weigh accurately an amount of Spiramycin II Acetate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spiramycin Acetate, 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. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spironolactone

スピロノラクトン



$C_{24}H_{32}O_4S$: 416.57

7α-Acetylsulfanyl-3-oxo-17α-pregn-4-ene-21,17-carbolactone [*52-01-7*]

Spironolactone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{24}H_{32}O_4S$.

Description Spironolactone occurs as a white to light yellow-brown, fine powder.

It is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol, and practically insoluble in water.

Melting point: 198 – 207°C (Insert the capillary tube into a bath at about 125°C, and continue the heating so that the temperature rises at a rate of about 10°C per minute in the range between 140°C and 185°C, and when the temperature is near the expected melting range, reduce the heating so that the temperature rises at a rate of about 3°C per minute.)

Identification **(1)** Determine the absorption spectrum of a solution of Spironolactone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Spironolactone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spironolactone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Spironolactone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Spironolactone and Spironolactone RS in methanol, respectively, then evaporate methanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -33 – $-37°$ (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

Purity **(1)** Mercapto compounds—Shake 2.0 g of Spironolactone with 20 mL of water, and filter. To 10 mL of the filtrate add 1 mL of starch TS and 0.05 mL of 0.01 mol/L iodine VS, and mix: a blue color develops.

(2) Related substances—Dissolve 0.20 g of Spironolactone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with *n*-butyl acetate to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in methanol (1 in 10) on the plate, and heat the plate at 105°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Spironolactone and Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, of the sample solution and standard solution at 238 nm.

$$\text{Amount (mg) of } C_{24}H_{32}O_4S = M_S \times A_T/A_S$$

M_S: amount (mg) of Spironolactone RS

Containers and storage Containers—Tight containers.

Spironolactone Tablets

スピロノラクトン錠

Spironolactone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$: 416.57).

Method of preparation Prepare as directed under Tablets,

with Spironolactone.

Identification To an amount of powdered Spironolactone Tablets, equivalent to 10 mg of Spironolactone according to the labeled amount, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 236 nm and 240 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Spironolactone Tablets add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 0.5 mg of spironolactone ($C_{24}H_{32}O_4S$). After stirring for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of spironolactone ($C_{24}H_{32}O_4S$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of Spironolactone RS

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 500 mL as the dissolution medium, the dissolution rate in 30 minutes of a 25-mg tablet and a 50-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Spironolactone Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 14 μg of spironolactone ($C_{24}H_{32}O_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in 20 mL of ethanol (95), and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of spironolactone ($C_{24}H_{32}O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Spironolactone RS
C: Labeled amount (mg) of spironolactone ($C_{24}H_{32}O_4S$) in 1 tablet

Assay Weigh accurately the mass of not less than 10 Spironolactone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of spironolactone ($C_{24}H_{32}O_4S$), add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. After stirring this solution for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of spironolactone from each solution.

Amount (mg) of spironolactone ($C_{24}H_{32}O_4S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Spironolactone RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside 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 methanol and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of spironolactone is about 11 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 the symmetry factor of the peak of spironolactone are not less than 4000 and not more than 1.5, respectively.

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

Containers and storage Containers—Tight containers.

Stearic Acid

ステアリン酸

Stearic Acid is solid fatty acids obtained from fats, and it consists chiefly of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Description Stearic Acid occurs as white, unctuous or crystalline masses or powder. It has a faint, fatty odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

Melting point: 56 – 72°C

Acid value <*1.13*> 194 – 210

Iodine value <*1.13*> Not more than 4.0.

Purity **(1)** Mineral acid—Melt 5 g of Stearic Acid by warming, shake with 5 mL of boiling water for 2 minutes, filter after cooling, and add 1 drop of methyl orange TS to the filtrate: no red color develops.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Stearic 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).

(3) Fat and paraffin—Boil 1.0 g of Stearic Acid with

0.5 g of anhydrous sodium carbonate and 30 mL of water: the solution, while hot, is clear or not more turbid than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 30 mL, and add 1 mL of silver nitrate TS.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Containers and storage Containers—Well-closed containers.

Stearyl Alcohol

ステアリルアルコール

Stearyl Alcohol is a mixture of solid alcohols, and consists chiefly of stearyl alcohol ($C_{18}H_{38}O$: 270.49).

Description Stearyl Alcohol occurs as a white, unctuous matter. It has a faint, characteristic odor. It is tasteless.

It is freely soluble in ethanol (95), in ethanol (99.5), in diethyl ether, and practically insoluble in water.

Melting point ⟨*1.13*⟩ 56 – 62°C under Melting Point Determination Prepare the sample according to Method 2 under Melting Point Determination, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

Acid value ⟨*1.13*⟩ Not more than 1.0.

Ester value ⟨*1.13*⟩ Not more than 3.0.

Hydroxyl value ⟨*1.13*⟩ 200 – 220

Iodine value ⟨*1.13*⟩ Not more than 2.0.

Purity **(1)** Clarity of solution—Dissolve 3.0 g of Stearyl Alcohol in 25 mL of ethanol (99.5) by warming: the solution is clear.

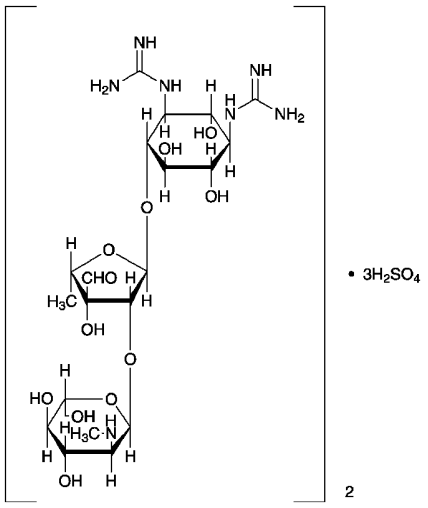
(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (2 g).

Containers and storage Containers—Well-closed containers.

Streptomycin Sulfate

ストレプトマイシン硫酸塩



$(C_{21}H_{39}N_7O_{12})_2.3H_2SO_4$: 1457.38
2-Deoxy-2-methylamino-α-L-glucopyranosyl-(1→2)-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-*N*,*N*′-diamidino-D-streptamine sesquisulfate
[*3810-74-0*]

Streptomycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces griseus*.

It contains not less than 740 μg (potency) and not more than 820 μg (potency) per mg, calculated on the dried basis. The potency of Streptomycin Sulfate is expressed as mass (potency) of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Description Streptomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and very slightly soluble in ethanol (95).

Identification **(1)** Dissolve 50 mg of Streptomycin Sulfate in 5 mL of water, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 10 minutes: a purple color is developed.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (7 in 100) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at about 150°C for about 5 minutes: the principal spots from the sample solution and the standard solution show the same in color tone and *R*f value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −79 – −88° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 2.0 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Streptomycin Sulfate in 5 mL of water is clear, and colorless or pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Streptomycin 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) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Streptomycin Sulfate according to Method 3 and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve exactly 0.20 g of Streptomycin Sulfate in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), add a mixture of methanol and sulfuric acid (97:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve exactly 36 mg of D(+)-mannose in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), and add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and sulfuric acid (97:3) 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and acetic acid (100) (2:1:1) to a distance of 13 to 15 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at 110°C for 5 minutes: the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Streptomycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), 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°C and 15°C, and use within 30 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 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Streptomycin Sulfate, equivalent to about 20 mg (potency), dissolve in water to make exactly 50 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 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Streptomycin Sulfate for Injection

注射用ストレプトマイシン硫酸塩

Streptomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Method of preparation Prepare as directed under Injections, with Streptomycin Sulfate.

Description Streptomycin Sulfate for Injection occurs as a white or light yellowish white masses or powder.

Identification Peform the test as directed in the Identification (2) under Streptomycin Sulfate.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 10 mL of water is 5.0 to 7.0.

Purity Clarity and color of solution—Dissolve an amount of Streptomycin Sulfate for Injection, equivalent to 1.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 3 mL of water: The solution is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.50.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.10 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate

method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organisms, culture medium and standard solutions—Proceed as directed in the Assay under Streptomycin Sulfate.

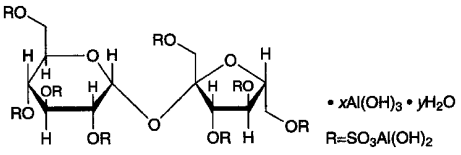
(ii) Sample solution—Weigh acculately the contents of not less than 10 Streptomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to 1 g (potency) of Streptomycin Sulfate, and dissolve in water to make exactly 200 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make a solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sucralfate Hydrate

Aluminum Sucrose Sulfate Ester

スクラルファート水和物



$C_{12}H_{30}Al_8O_{51}S_8.xAl(OH)_3.yH_2O$
[*54182-58-0*]

Sucralfate Hydrate contains not less than 17.0% and not more than 21.0% of aluminum (Al: 26.98) and not less than 34.0% and not more than 43.0% of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$: 982.80), calculated on the dried basis.

Description Sucralfate Hydrate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in hot water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid-sodium hydroxide TS.

Identification **(1)** To 0.05 g of Sucralfate Hydrate in a small test tube add 0.05 g of fresh pieces of sodium, and melt by careful heating. Immerse the test tube immediately in 100 mL of water, break the test tube, shake well, and filter. To 5 mL of the filtrate add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

(2) Dissolve 40 mg of Sucralfate Hydrate in 2 mL of dilute sulfuric acid, and add gently 2 mL of anthrone TS to make 2 layers: a blue color develops at the zone of contact, and gradually changes to blue-green.

(3) Dissolve 0.5 g of Sucralfate Hydrate in 10 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for aluminum.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sucralfate Hydrate in 10 mL of dilute sulfuric acid: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Sucralfate Hydrate in 30 mL of dilute nitric acid, and heat gently to boiling. After cooling, add water to make 100 mL, and to 10 mL of this solution add 3 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 (not more than 0.50%).

(3) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Sucralfate Hydrate in 20 mL of a solution of sodium chloride (1 in 5) and 1 mL of dilute hydrochloric acid, and to this solution 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: evaporate 1 mL of dilute hydrochloric acid on a water bath to dryness, and add 20 mL of a solution of sodium chloride (1 in 5), 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Arsenic ⟨*1.11*⟩—Dissolve 1.0 g of Sucralfate Hydrate in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Free aluminum—To 3.0 g of Sucralfate Hydrate add 50 mL of water, heat in a water bath for 5 minutes, cool, and filter. Wash the residue with four 5-mL portions of water, combine the filtrate with the washings, add 2 mL of dilute hydrochloric acid, and heat in a water bath for 30 minutes. After cooling, neutralize the solution with sodium hydroxide TS, add water to make exactly 100 mL, and use this solution as the sample solution. Pipet 50 mL of the sample solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination (not more than 0.2%).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.349 mg of Al

(6) Related substances—Proceed with 50 μL of the sample solution obtained in the Assay (2) Sucrose octasulfate ester as directed in the Assay (2) Sucrose octasulfate ester, and perform the test as directed under Liquid Chromatography ⟨*2.01*⟩. Determine the peak area of sucrose octasulfate ester from the sample solution and that of a related substance with the relative retention time about 0.7 to the peak of sucrose octasulfate ester by the automatic integration method, and calculate the ratio of the peak area of the related substance to that of sucrose octasulfate ester: it is not more than 0.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of sucrose octasulfate ester from 50 μL of the standard solution obtained in the Assay (2) Sucrose octasulfate ester composes 60% to 100% of the full scale.

Loss on drying ⟨*2.41*⟩ Not more than 14.0% (1 g, 105°C, 3 hours).

Acid-consuming capacity Weigh accurately about 0.25 g of Sucralfate Hydrate, previously dried, place in a 200-mL glass-stoppered conical flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, and shake at 37 ± 2°C for exactly 1 hour (150 shakings per

minute, amplitude: 20 mm). After cooling in water for 5 minutes, pipet 10 mL of the supernatant liquid, and titrate ⟨*2.50*⟩ the excess acid with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. Perform a blank determination. The amount of 0.1 mol/L hydrochloric acid VS consumed per g of Sucralfate is not less than 130 mL.

Assay (1) Aluminum—Weigh accurately about 1 g of Sucralfate Hydrate, dissolve in 10 mL of dilute hydrochloric acid by warming on a water bath, cool, and add water to make exactly 250 mL. Pipet 25 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.349 mg of Al

(2) Sucrose octasulfate ester—Weigh accurately about 0.55 g of Sucralfate Hydrate, add exactly 10 mL of sulfuric acid-sodium hydroxide TS, shake vigorously, and dissolve with ultrasonic wave at below 30°C for 5 minutes. To this solution add 0.1 mol/L sodium hydroxide VS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Potassium Sucrose Octasulfate RS, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Prepare rapidly the sample solution and the standard solution, and perform the test immediately. Pipet 50 μL each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_T and A_S, of sucrose octasulfate ester from each solution.

Amount (mg) of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$)
$= M_S \times A_T/A_S \times 0.763$

M_S: Amount (mg) of Potassium Sucrose Octasulfate RS, calculated on the anhydrous basis

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (about 8 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve a suitable amount (26 to 132 g) of ammonium sulfate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and perform the test immediately. Adjust the amount of ammonium sulfate in the mobile phase so that the peak of a related substance with the relative retention time being about 0.7 to that of sucrose octasulfate ester almost returns to the base line, and the peak of sucrose octasulfate ester elutes most rapidly.

Flow rate: Adjust the flow rate so that the retention time of sucrose octasulfate ester is between 6 and 11 minutes.

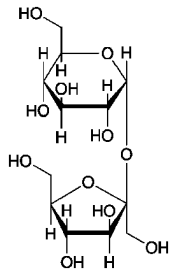
Selection of column: Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and proceed immediately with 50 μL of this solution under the above operating conditions. Use a column with a resolution being not less than 1.5 between sucrose octasulfate ester and a related substance with the relative retention time being about 0.7 to sucrose octasulfate ester.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of sucrose octasulfate ester is not more than 2.0%.

Containers and storage Containers—Tight containers.

Sucrose

精製白糖



$C_{12}H_{22}O_{11}$: 342.30
β-D-Fructofuranosyl α-D-glucopyranoside
[*57-50-1*]

Sucrose contains no additives.

For Sucrose used for preparation of the large volume infusions, the label states the purpose.

Description Sucrose is a white crystalline powder, or lustrous colorless or white crystals.

It is very soluble in water, and slightly soluble in ethanol (95).

Identification (1) To 10 mg each of Sucrose and white soft sugar add diluted methanol (3 in 5) to make 20 mL each, and use these solutions as the sample solution and the standard solution (a), respectively. Separately, to 10 mg each of glucose, lactose monohydrate, fructose and white soft sugar add methanol (3 in 5) to make 20 mL, and use this solution as the standard solution (b). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solutions (a) and (b) on a plate of silica gel for thin-layer chromatography, and dry the plate completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a hot air. And immediately repeat the development with replaced developing mixture, and dry the plate in the same way. Spray evenly a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), heat at 130°C for 10 minutes: the principal spot from the sample solution is the same with the principal spot from the standard solution (a) in the *R*f value, color and size, and

four spots from the standard solution (b) are apparently distinguishable.

(2) Dissolve 50.0 g of Sucrose in recently boiled and cooled water to make 100 mL, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 100 mL, then to 5 mL of this solution add 0.15 mL of freshly prepared copper (II) sulfate TS and 2 mL of freshly prepared 2 mol/L sodium hydroxide TS: the solution is clear and blue, and not changes on boiling. Then to this solution add 4 mL of dilute hydrochloric acid, boil, and add 4 mL of 2 mol/L sodium hydroxide TS: orange precipitates are immediately produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +66.3 – +67.0° (26 g, water, 100 mL, 100 mm).

Purity **(1)** Clarity and color of solution—The sample solution obtained in the Identification (2) is clear, and has no more color than the following control solution.

Control solution: To exactly 2.4 mL of Iron (III) Chloride CS and exactly 0.6 mL of Cobalt (II) Chloride CS add 7.0 mL of diluted hydrochloric acid (7 in 250). To 5.0 mL of this solution add 95.0 mL of diluted hydrochloric acid (7 in 250).

(2) Acidity or alkalinity—To 10 mL of the sample solution obtained in the Identification (2) add 0.3 mL of phenolphthalein TS: the solution is colorless, and develops a red color on addition of 0.3 mL of 0.01 mol/L sodium hydroxide TS.

(3) Sulfite—Dissolve 5.0 g of Sucrose in 40 mL of water, add 2.0 mL of dilute sodium hydroxide TS and water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 76 mg of sodium disulfite in water to make exactly 50 mL, then pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Immediately, pipet 10 mL each of the sample solution and the standard solution, add 1.0 mL of 3 mol/L hydrochloric acid TS, 2.0 mL of decolorized fuchsin TS and 2.0 mL of formaldehyde solution TS, and allow to stand for 30 minutes. Determine the absorbance at 583 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using the control solution obtained by proceeding with 10.0 mL of water in the same manner as above: the absorbance of the sample solution is not larger than that of the standard solution (not more than 15 ppm as SO_2). When the standard solution does not show a red-purple to blue-purple color, result of the test is invalid.

(4) Lead—Put exactly 50 mg of Sucrose in a polytetrafuruoroethylene decomposition-vessel, add 0.5 mL of nitric acid to dissolve, seal up the vessel, and heat at 150°C for 5 hours. After cooling, add water to make exactly 5 mL, and use this solution as the sample solution. Perform the test with more than 3 parts of the sample solution as directed in the standard addition method under Atomic Absorption Spectrophotometry <2.23> (electrothermal type) according to the following conditions. The standard solution is prepared by adding water to a suitable volume of Standard Lead Solution exactly volumed, and perform a blank determination with a solution prepared by adding water to 10.0 mL of nitric acid to make exactly 100 mL, and make any necessary correction (not more than 0.5 ppm).

Operating conditions—

Lamp: A hollow cathode lamp.

Wavelengh: 283.3 mm.

Temperature for drying: 110°C.

Temperature for incineration: 600°C.

Temperature for atomization: 2100°C.

(5) Invert sugar—Transfer 5 mL of the sample solution obtained in the Identification (2) to a test-tube about 150 mm long and about 16 mm in diameter, add 5 mL of water, 1.0 mL of 1 mol/L sodium hydroxide VS and 1.0 mL of methylene blue TS, mix, and place in a water bath. After exactly 2 minutes, take the tube out of the bath, and examine the solution immediately: the blue color does not disappear completely (0.04%). Ignore any blue color at the air and solution interface.

Conductivity

(i) Potassium chloride conductivity calibration standard solution—Dissolve powdered potassium chloride, previously dried at 500 – 600°C for 4 hours, in newly distillated water having less conductivity than 2 $\mu S \cdot cm^{-1}$ to get three kinds of the standard solution containing 0.7455 g, 0.0746 g and 0.0149 g of potassium chloride in 1000.0 g, respectively. The conductivities of these solutions at 20°C are shown in the following table.

Standard solution (g/1000.0 g)	Conductivity ($\mu S \cdot cm^{-1}$)	Resistivity ($\Omega \cdot cm$)
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

(ii) Apparatus—Use an appropriate conductivity meter. The conductivity is determined to measure the electrical resistance of the column of liquid between the electrodes of the immersed measuring device (conductivity cell). The apparatus is supplied with alternative current to avoid the effects of electrode polarization. It is usually equipped with a temperature compensation device. The conductivity cell contains of two parallel platinum electrodes coated with platinum black, and both electrodes are generally protected by a glass tube which allows good exchange between the solution and the electrodes. Use a cell giving the cell constant of 0.01 to 1 cm^{-1}.

(iii) Procedure—Use the suitable potassium chloride conductivity calibration standard solution to the measurement. After washing the well with water, rinse 2 to 3 times with the calibration standard solution, fill up the cell with the calibration standard solution, and determine the conductivity of the calibration standard solution kept at 20 ± 0.1°C. Repeat the determination, and measure the conductivity of the calibration standard solution, G_{χ_0} (μS), after a stable reading of ± 3% is obtained. The cell constant, J, is calculated by the following:

$$J = \frac{\chi_{KCl}}{G_{\chi_0}}$$

J: Cell constant (cm^{-1})

χ_{KCl}: Conductivity constant of the potassium chloride conductivity calibration standard solution ($\mu S \cdot cm^{-1}$) (20°C)

G_{χ_0}: Conductivity measured (μS)

Dissolve 31.3 g of Sucrose in newly distillated water to make exactly 100 mL, and use this solution as the sample so-

lution. After washing well the cell with water, rinse the cell with the sample solution 2 to 3 times , fill up with the sample solution, and determine the conductivity of the sample solution, G_T (μS), kept at 20 ± 0.1°C, while stirring. Determine the conductivity of the water used for preparation of the sample solution, G_0 (μS), in the same manner as above, and calculate the conductivity, χ_T ($\mu S \cdot cm^{-1}$) and χ_0 ($\mu S \cdot cm^{-1}$), by the following expressions:

$$\chi_T\ (\mu S \cdot cm^{-1}) = JG_T$$
$$\chi_0\ (\mu S \cdot cm^{-1}) = JG_0$$

Determine the corrected conductivity, χ_C, of the sample solution by the following expression: not more than 35 $\mu S \cdot cm^{-1}$.

$$\chi_C\ (\mu S \cdot cm^{-1}) = \chi_T - 0.35\,\chi_0$$

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (2 g, 105°C, 3 hours).

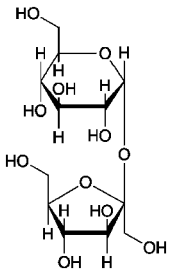
Dextrins For Sucrose used to prepare large volume infusions, to 2 mL of the sample solution obtained in the Identification (2) add 8 mL of water, 0.05 mL of dilute hydrochloric acid and 0.05 mL of iodine TS: the solution remains yellow.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mg, for Sucrose exclusively to be used to prepare Injections for intravenous infusion of larger volume.

Containers and storage Containers—Well-closed containers.

White Soft Sugar

白糖



$C_{12}H_{22}O_{11}$: 342.30
β-D-Fructofuranosyl α-D-glucopyranoside
[*57-50-1*]

Description White Soft Sugar is colorless or white crystals or crystalline powder. It is odorless and has a sweet taste.

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

A solution of White Soft Sugar (1 in 10) is neutral.

Identification **(1)** When 1 g of White Soft Sugar is ignited, it melts and swells, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of White Soft Sugar add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark red precipitate is produced.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +65.0 – +67.0° (after drying, 13 g, water, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 100 g of White Soft Sugar in 100 mL of water, take 50 mL of this solution in a Nessler tube, and view transversely the Nessler tube against a white background: the solution is colorless or only slightly yellow and has no blue color. Fill the solution in the Nessler tube, stopper, and allow to stand for 2 days: no precipitate is produced.

(2) Chloride ⟨*1.03*⟩—To 10.0 g of White Soft Sugar add water to make 100 mL, and use this solution as the sample solution. To 20 mL of the sample 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 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate ⟨*1.14*⟩—To 40 mL of the sample solution 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 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Calcium—To 10 mL of the sample solution obtained in (2) add 1 mL of ammonium oxalate TS: this solution shows immediately no change.

(5) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of White Soft Sugar 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).

(6) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of White Soft Sugar according to Method 1, and perform the test (not more than 2 ppm).

(7) Invert sugar—Dissolve 5.0 g of White Soft Sugar in water to make 100 mL, filter if necessary, and use this solution as the sample solution. Separately place 100 mL of alkaline copper (II) sulfate solution in a 300-mL beaker, cover the beaker with a watch glass, and boil. Immediately add 50.0 mL of the sample solution, boil the mixture exactly for 5 minutes, add at once 50 mL of freshly boiled and cooled water, dip it in a water bath of a temperature below 10°C for 5 minutes, and collect the precipitate in a tared glass filter (G4). Wash the residue on the filter with water until the last washing is neutral, then wash with 10 mL of ethanol (95), add 10 mL of diethyl ether, and dry at 105°C for 30 minutes: the mass of the residual precipitate is not more than 0.120 g.

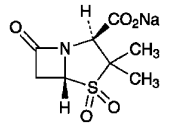
Loss on drying ⟨*2.41*⟩ Not more than 1.30% (15 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (2 g).

Containers and storage Containers—Well-closed containers.

Sulbactam Sodium

スルバクタムナトリウム



$C_8H_{10}NNaO_5S$: 255.22
Monosodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide
[*69388-84-7*]

Sulbactam Sodium contains not less than 875 μg (potency) and not more than 941 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbactam Sodium is expressed as mass (potency) of sulbactam ($C_8H_{11}NO_5S$: 233.24).

Description Sulbactam Sodium occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification **(1)** Determine the infrared absorption spectrum of Sulbactam Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbactam Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +219 – +233° (1 g, water, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the pH of the solution is between 5.2 and 7.2.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the solution is clear, and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Sulbactam 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 <*1.11*>—Prepare the test solution with 1.0 g of Sulbactam Sodium as directed in Method 3, and perform the test (not more than 2 ppm).

(4) Sulbactam penicillamine—Weigh accurately about 0.2 g of Sulbactam Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL of water, add 0.5 mL of sodium hydroxide TS, allow to stand for 10 minutes at a room temperature, and add 0.5 mL of 1 mol/L hydrochloric acid TS, then add the mobile phase 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of sulbactam penicillamine by the automatic integration method: the amount of sulbactam penicillamine is not more than 1.0%.

$$\text{Amount (\%) of sulbactam penicillamine} = M_S/M_T \times A_T/A_S \times 5$$

M_S: Amount (mg) of sulbactam sodium for sulbactam penicillamine
M_T: Amount (mg) of the sample

Operating conditions—
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
System suitability—
System performance: Proceed as directed in the system suitability in the Assay.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam penicillamine is not more than 2.0%.

Water <*2.48*> Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Sulbactam Sodium and Sulbactam RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sulbactam to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of sulbactam } (C_8H_{11}NO_5S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Sulbactam RS

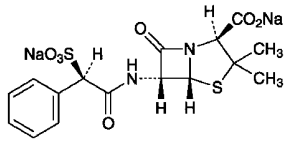
Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust the flow rate so that the retention time of sulbactam 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, sulbactam 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sulbenicillin Sodium

スルベニシリンナトリウム



$C_{16}H_{16}N_2Na_2O_7S_2$: 458.42
Disodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*R*)-2-phenyl-2-sulfonatoacetylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*28002-18-8*]

Sulbenicillin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbenicillin Sodium is expressed as mass (potency) of sulbenicillin ($C_{16}H_{18}N_2O_7S_2$: 414.45).

Description Sulbenicillin Sodium occurs as white to light yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Sulbenicillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Sulbenicillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +167 – +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.20 g of Sulbenicillin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 2.5 g of Sulbenicillin Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Sulbenicillin 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 <*1.11*>—Prepare the test solution with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulbenicillin Sodium in 15 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the each peak other than the two peaks of sulbenicillin is not more than 2.0%, and the total amount of the peaks other than the two peaks of sulbenicillin is not more than 5.0%.

Operating conditions—

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

Column: A stainless steel column 3.9 mm in inside diameter and 30 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 10 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 ± 0.1 with sodium hydroxide TS, and add water to make 1000 mL. To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of the lately eluted peak of sulbenicillin is about 18 minutes.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak of sulbenicillin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the resolution between the two peaks of sulbenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total areas of the two peaks of sulbenicillin is not more than 5.0%.

Water <*2.48*> Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.4 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Sulbenicillin Sodium RS, equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a freezer, and use within 4 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as

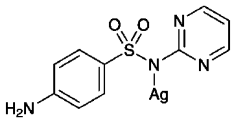
the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sulbenicillin Sodium, equivalent to about 50 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sulfadiazine Silver

スルファジアジン銀



$C_{10}H_9AgN_4O_2S$: 357.14
Monosilver 4-amino-*N*-(pyrimidin-2-yl)-benzenesulfonamidate
[*22199-08-2*]

Sulfadiazine Silver, when dried, contains not less than 99.0% and not more than 102.0% of $C_{10}H_9AgN_4O_2S$.

Description Sulfadiazine Silver occurs as a white to pale yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in ammonia TS.

It is gradually colored by light.

Melting point: about 275°C (with decomposition).

Identification Determine the infrared absorption spectrum of Sulfadiazine Silver, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Sulfadiazine Silver RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Nitrate—To 250 mL of water add 1.0 g of Sulfadiazine Silver, shake well for 50 minutes, filter, and use this filtrate as the sample solution. Separately, weigh accurately 0.25 g of potassium nitrate, and dissolve in water to make exactly 2000 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 2.0 mL each of the sample solution and the standard solution, and add 5 mL of a solution of cromotropic acid in sulfuric acid (1 in 10,000) and sulfuric acid to make exactly 10 mL. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with exactly 2.0 mL of water in the same manner, as the blank: A_T is not larger than A_S (not more than 0.05%).

(2) Related substances—Dissolve 50 mg of Sulfadiazine Silver in 5 mL of a mixture of ethanol (95) and ammonia solution (28) (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of ethanol (95) and ammonia solution (28) (3:2) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (3:2) 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 ⟨*2.03*⟩. Spot 5 μL each of the sample 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, methanol and ammonia solution (28) (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 other than the principal spot and spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80%, 4 hours).

Residue on ignition ⟨*2.44*⟩ 41 – 45% (1 g).

Silver content Weigh accurately about 50 mg of Sulfadiazine Silver, previously dried, dissolve in 2 mL of nitric acid, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Measure accurately a suitable quantity of Standard Silver Solution for Atomic Absorption Spectrophotometry, dilute with water to make solutions containing 1.0 to 2.0 μg of silver (Ag:107.87) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions, and calculate the silver content of the sample solution from the calibration curve obtained from the absorbances of the standard solutions: it contains not less than 28.7% and not more than 30.8% of silver.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: A silver hollow cathode lamp.
Wavelength: 328.1 nm.

Assay Weigh accurately about 0.1 g each of Sulfadiazine Silver and Sulfadiazine Silver RS, each previously dried, and add ammonia TS to make exactly 100 mL, respectively. Pipet 1 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 255 nm, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with exactly 1 mL of ammonia TS and a sufficient water to make exactly 100 mL, as the blank.

$$\text{Amount (mg) of } C_{10}H_9AgN_4O_2S = M_S \times A_T/A_S$$

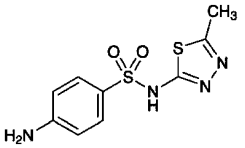
M_S: Amount (mg) of Sulfadiazine Silver RS

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamethizole

スルファメチゾール



$C_9H_{10}N_4O_2S_2$: 270.33
4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-benzenesulfonamide
[*144-82-1*]

Sulfamethizole, when dried, contains not less than 99.0% of $C_9H_{10}N_4O_2S_2$.

Description Sulfamethizole occurs as white to yellowish white crystals or crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), and in acetic acid (100) and practical insoluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethizole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 208 – 211°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Sulfamethizole in 3 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethizole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand for 1 hour in an ice bath, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Sulfamethizole 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Sulfamethizole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sulfamethizole in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 50 mL, then 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 these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample 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 and acetic acid (100) (20:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethizole, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium nitrite VS according to the potentiometric titration method or the amperometric titration method.

Each mL of 0.1 mol/L sodium nitrite VS
= 27.03 mg of $C_9H_{10}N_4O_2S_2$

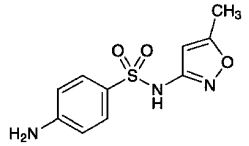
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamethoxazole

Sulfisomezole

スルファメトキサゾール



$C_{10}H_{11}N_3O_3S$: 253.28
4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide
[*723-46-6*]

Sulfamethoxazole, when dried, contains not less than 99.0% of $C_{10}H_{11}N_3O_3S$.

Description Sulfamethoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in *N*,*N*-dimethylformamide, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethoxazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 169 – 172°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sulfamethoxazole in 5 mL of sodium hydroxide TS, and add 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethoxazole add 50 mL of water, heat at 70°C 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 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Sul-

famethoxazole 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 <1.11>—Prepare the test solution with 1.0 g of Sulfamethoxazole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Sulfamethoxazole in 10 mL of a solution of ammonia solution (28) in methanol (1 in 50), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 10 mL. Pipet 1 mL of this solution, add a solution of ammonia solution (28) in methanol (1 in 50) 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 <2.03>. Spot 5 μL each of the sample 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, acetonitrile and diluted ammonia solution (28) (7 in 100) (10:8:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethoxazole, previously dried, dissolve in 30 mL of *N*,*N*-dimethylformamide, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light blue color is produced (indicator: 0.5 mL of thymolphthalein TS). Separately, perform a blank determination in the same manner with a mixture of 30 mL of *N*,*N*-dimethylformamide and 26 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.33 mg of $C_{10}H_{11}N_3O_3S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamonomethoxine Hydrate

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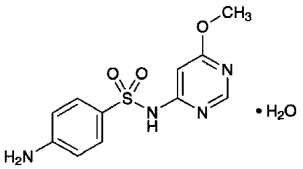


$C_{11}H_{12}N_4O_3S.H_2O$: 298.32
4-Amino-*N*-(6-methoxypyrimidin-4-yl)benzenesulfonamide Monohydrate
[*1220-83-3*, anhydride]

Sulfamonomethoxine Hydrate, when dried, contains not less than 99.0% of sulfamonomethoxine ($C_{11}H_{12}N_4O_3S$: 280.31).

Description Sulfamonomethoxine Hydrate occurs as white to pale yellow crystals, granules or crystalline powder. It is odorless.

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

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamonomethoxine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 204 – 206°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow. Dissolve 0.5 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS, and heat: no turbidity is produced. After cooling, add 5 mL of acetone: the solution is clear.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sulfamonomethoxine 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 <1.11>—Prepare the test solution with 1.0 g of Sulfamonomethoxine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.02 g of Sulfamonomethoxine Hydrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 <2.03>. Spot 5 μL each of the sample 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-butanol and ammonia solution (28) (4:1) 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 sample solution are not larger and not more intense than the spot from the standard solution.

Loss on drying <2.41> 4.5 – 6.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Sulfamonomethoxine Hydrate, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 28.03 mg of $C_{11}H_{12}N_4O_3S$

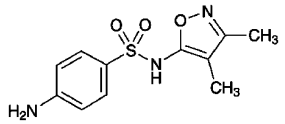
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfisoxazole

Sulfafurazole

スルフイソキサゾール



$C_{11}H_{13}N_3O_3S$: 267.30
4-Amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide
[*127-69-5*]

Sulfisoxazole, when dried, contains not less than 99.0% of $C_{11}H_{13}N_3O_3S$.

Description Sulfisoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in pyridine and in *n*-butylamine, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), and very slightly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Sulfisoxazole in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(2) Dissolve 0.02 g of Sulfisoxazole in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Add 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(3) Dissolve 0.01 g of Sulfisoxazole in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfisoxazole add 2 mL of acetic acid (100), dissolve by heating under a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, and alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10). Filter, if necessary, immediately acidify by adding acetic acid (100) dropwise, collect the produced precipitate, recrystallize from methanol, and dry at 105°C for 1 hour: the crystals melt <*2.60*> between 208°C and 210°C.

Melting point <*2.60*> 192 – 196°C (with decomposition).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sulfisoxazole in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity—To 1.0 g of Sulfisoxazole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand in an ice bath for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Sulfisoxazole 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 <*2.41*> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Sulfisoxazole, previously dried, dissolve in 50 mL of methanol by warming, cool and titrate <*2.50*> with 0.2 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination using a mixture of 50 mL of methanol and 18 mL of water, and make any necessary correction.

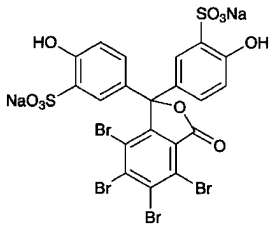
Each mL of 0.2 mol/L sodium hydroxide VS
= 53.46 mg of $C_{11}H_{13}N_3O_3S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfobromophthalein Sodium

スルホブロモフタレインナトリウム



$C_{20}H_8Br_4Na_2O_{10}S_2$: 838.00
Disodium 5,5′-(4,5,6,7-tetrabromo-3-oxo-1,3-dihydroisobenzofuran-1,1-diyl)bis(2-hydroxybenzenesulfonate)
[*71-67-0*]

Sulfobromophthalein Sodium, when dried, contains not less than 96.0% and not more than 104.0% of $C_{20}H_8Br_4Na_2O_{10}S_2$.

Description Sulfobromophthalein Sodium occurs as a white, crystalline powder. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification **(1)** Dissolve 0.02 g of Sulfobromophthalein Sodium in 10 mL of water, and add 1 mL of sodium carbonate TS: a blue-purple color is produced. Add 1 mL of dilute hydrochloric acid to the solution: the color of the solution disappears.

(2) Transfer 0.2 g of Sulfobromophthalein Sodium to a porcelain crucible, mix well with 0.5 g of anhydrous sodium carbonate, and ignite until the mixture is charred. After cooling, add 15 mL of hot water to the residue, heat for 5 minutes on a water bath, filter, and render the filtrate slightly acid with hydrochloric acid: the solution responds to the Qualitative Tests <*1.09*> for bromide, and (1) and (2) for sulfate.

(3) Sulfobromophthalein Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH ⟨*2.54*⟩ The pH of a solution of 1.0 g of Sulfobromophthalein Sodium in 20 mL of water is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Sulfobromophthalein Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Sulfobromophthalein Sodium. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Sulfate—To 10 mL of a solution of Sulfobromophthalein Sodium (1 in 500) add 5 drops of dilute hydrochloric acid, heat to boil, and add 1 mL of hot barium chloride TS: the solution is clear when observed 1 minute after the addition of the barium chloride TS.

(4) Calcium—Weigh accurately about 5 g of Sulfobromophthalein Sodium, transfer to a porcelain dish, heat gently to char, and heat strongly between 700°C and 750°C until the residue is incinerated. After cooling, add 10 mL of dilute hydrochloric acid, and heat for 5 minutes on a water bath. Transfer the contents to a flask with 50 mL of water, and add 5 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator. Titrate ⟨*2.50*⟩ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.4008 mg of Ca

The content of calcium (Ca: 40.08) is not more than 0.05%.

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Sulfobromophthalein 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).

(6) Arsenic ⟨*1.11*⟩—Transfer 0.65 g of Sulfobromophthalein Sodium to a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), fire to burn, then heat gently until the residue is incinerated. If any carbon remains, moisten the residue with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 10 mL of dilute sulfuric acid, and heat until white fumes are evolved. After cooling, add 5 mL of water to the residue, and perform the test with this solution as the test solution (not more than 3.1 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ 14 – 19% (after drying, 0.5 g, 700 – 750°C).

Assay Dissolve about 0.1 g of Sulfobromophthalein Sodium, previously dried and accurately weighed, in water to make exactly 500 mL. Pipet 5 mL of this solution, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 580 nm, using water as the blank.

Amount (mg) of $C_{20}H_8Br_4Na_2O_{10}S_2$
$= A/881 \times 200{,}000$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sulfobromophthalein Sodium Injection

スルホブロモフタレインナトリウム注射液

Sulfobromophthalein Sodium Injection is an aqueous solution for Injection.

It contains not less than 94.0% and not more than 106.0% of the labeled amount of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$: 838.00).

Method of preparation Prepare as directed under Injections, with Sulfobromophthalein Sodium.

Description Sulfobromophthalein Sodium Injection is a clear and colorless or pale yellow liquid.

pH: 5.0 – 6.0

Identification **(1)** Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.02 g of Sulfobromophthalein Sodium according to the labeled amount, and proceed as directed in the Identification (1) under Sulfobromophthalein Sodium.

(2) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.1 g of Sulfobromophthalein Sodium according to the labeled amount, add 0.5 g of anhydrous sodium carbonate, and evaporate on a water bath to dryness. Ignite the residue until it is charred. Proceed as directed in the Identification (2) under Sulfobromophthalein Sodium.

Pyrogen ⟨*4.04*⟩ Add isotonic sodium chloride solution to Sulfobromophthalein Sodium Injection to make a 0.5 w/v% solution of Sulfobromophthalein Sodium according to the labeled amount. Inject into each of the rabbits 5 mL of this solution per kg of body mass: it meets the requirement.

Extractble volume ⟨*6.05*⟩ It meets the repequirement.

Assay Measure exactly a volume of Sulfobromophthalein Sodium Injection, equivalent to about 0.1 g of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$), add water to make exactly 500 mL, and proceed as directed in the Assay under Sulfobromophthalein Sodium.

Amount (mg) of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$)
$= A/881 \times 200{,}000$

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Sulfur

イオウ

S: 32.07

Sulfur, when dried, contains not less than 99.5% of S.

Description Sulfur occurs as a light yellow to yellow powder. It is odorless and tasteless.

It is freely soluble in carbon disulfide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification **(1)** Ignite Sulfur: it burns with a blue flame and gives a pungent odor of sulfur dioxide.

(2) Dissolve 5 mg of Sulfur in 5 mL of sodium hydroxide TS by heating in a water bath, cool, and add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a blue-purple color develops.

(3) Boil 1 mg of sulfur with 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS: a blue color develops.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Sulfur in a mixture of 20 mL of a solution of sodium hydroxide (1 in 6) and 2 mL of ethanol (95) by boiling: the solution is clear. Dissolve 2.0 g of Sulfur in 10 mL of carbon disulfide: the solution is almost clear or slightly opalescent.

(2) Acidity or alkalinity—Shake 2.0 g of Sulfur with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops. Further add 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Arsenic <*1.11*>—Prepare the test solution with 0.20 g of Sulfur according to Method 3, and perform the test (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, not more than 0.67 kPa, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.4 g of Sulfur, previously dried, dissolve in 20 mL of potassium hydroxide-ethanol TS and 10 mL of water by boiling, cool, and add water to make exactly 100 mL. Transfer exactly 25 mL of the solution to a 400-mL beaker, add 50 mL of hydrogen peroxide TS, and heat on a water bath for 1 hour. Acidify the solution with dilute hydrochloric acid, add 200 mL of water, heat to boil, add hot barium chloride TS dropwise until no more precipitate is formed, and heat on a water bath for 1 hour. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, heat strongly to constant mass, and weigh as barium sulfate ($BaSO_4$: 233.39). Perform a blank determination, and make any necessary correction.

Amount (mg) of S
= amount (mg) of barium sulfate ($BaSO_4$) × 0.13739

Containers and storage Containers—Well-closed containers.

Sulfur and Camphor Lotion

イオウ·カンフルローション

Method of preparation

Sulfur	60 g
d-Camphor or *dl*-Camphor	5 g
Hydroxypropylcellulose	4 g
Calcium Hydroxide	1 g
Ethanol	4 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve Hydroxypropylcellulose in 200 mL of Water, Purified Water or Purified Water in Containers. Add this solution in small portions to the triturate of Sulfur with the Ethanol solution of *d*-Camphor or *dl*-Camphor, and triturate again the mixture. Separately, dissolve Calcium Hydroxide in 500 mL of Water, Purified Water or Purified Water in Containers, stopper tightly, shake, and allow to stand. Add 300 mL of this supernatant liquid to the above mixture, then add Water, Purified Water or Purified Water in Containers to make 1000 mL, and shake thoroughly.

Description Sulfur and Camphor Lotion is a light yellow suspension.

A part of the components separates out on standing.

Identification **(1)** To 5 mL of well shaken Sulfur and Camphor Lotion add 25 mL of water, and centrifuge [use this supernatant liquid for test (3)]. To 0.02 g of the precipitate add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a blue color develops (sulfur).

(2) To 10 mL of well shaken Sulfur and Comphor Lotion add 5 mL of diethyl ether, and mix. Separate the diethyl ether layer, and filter through a pledget of cotton. Wash the cotton with a small portion of diethyl ether, combine the washings with the filtrate, and distil cautiously on a water bath to remove the diethyl ether. Dissolve the residue in 1 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for about 2 minutes on a water bath. Cool, dilute with water to make about 5 mL, and allow to stand. Filter the produced precipitate through a glass filter (G4), and wash the residue on the filter with water until the last washing is colorless. Dissolve the residue in 10 mL of ethanol (95), add 5 mL of sodium hydroxide TS, and allow to stand for 2 minutes: a red color develops (*d*-camphor or *dl*-camphor).

(3) The supernatant liquid obtained in (1) responds to the Qualitative Tests <*1.09*> (2) and (3) for calcium salt.

Containers and storage Containers—Tight containers.

Sulfur, Salicylic Acid and Thianthol Ointment

イオウ·サリチル酸·チアントール軟膏

Method of preparation

Sulfur	100 g
Salicylic Acid, finely powdered	30 g
Thianthol	100 mL
Zinc Oxide, very finely powdered	100 g
Simple Ointment or a suitable ointment base	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with above ingredients.

Description Sulfur, Salicylic Acid and Thianthol Ointment is light yellow in color.

Identification **(1)** Stir well 0.5 g of Sulfur, Salicylic Acid and Thianthol Ointment with 10 mL of water while heating, cool, and filter. To 1 mL of the filtrate add 5 mL of iron (III) nitrate TS: a purple color is produced (salicylic acid).

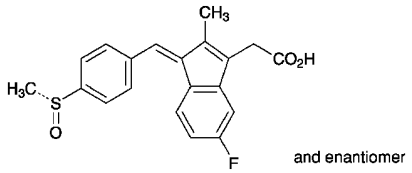
(2) Shake 1 g of Sulfur, Salicylic Acid and Thianthol Ointment with 20 mL of diethyl ether, remove the supernatant liquid and floating materials. Wash the residue with 10 mL of diethyl ether, and remove the diethyl ether by suction. To the residue add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a light blue to blue color is produced (sulfur).

(3) To 1 g of Sulfur, Salicylic Acid and Thianthol Ointment add 15 mL of ethanol (95), stir well while warming on a water bath, cool, and filter. Use the filtrate as the sample solution. Dissolve 0.01 g each of salicylic acid and thianthol in 5 mL of ethanol (95), and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots of each component obtained from the sample solution and standard solutions (1) and (2) show the same *R*f value. Spray iron (III) chloride TS upon the plate evenly: the spot from the standard solution (1) and that from the corresponding sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Sulindac

スリンダク



$C_{20}H_{17}FO_3S$: 356.41

(1*Z*)-(5-Fluoro-2-methyl-1-{4-[(*RS*)-methylsulfinyl]benzylidene}-1*H*-inden-3-yl)acetic acid [*38194-50-2*]

Sulindac, when dried, contains not less than 99.0% and not more than 101.0% of $C_{20}H_{17}FO_3S$.

Description Sulindac occurs as a yellow, crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Sulindac in methanol (1 in 100) shows no optical rotation.

Melting point: about 184°C (with decomposition).

Identification **(1)** Dissolve 15 mg of Sulindac in 1000 mL of a solution of hydrochloric acid in methanol (1 in 120). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry <*2.24*> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sulindac as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Sulindac 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 <*1.11*>—Prepare the test solution with 1.0 g of Sulindac according to Method 3 and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.25 g of Sulindac in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution and add methanol to make exactly 100 mL. Pipet 5 mL, 4 mL and 2 mL of this solution, to each add methanol to make exactly 10 mL, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 4 μL each of the sample solution, standard solution (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (97:3) to a distance of about 17 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the total intensity of spots other than the principal spot from the sample solution is not more than 1.0% calculated

on the basis of intensities of the spots from the standard solution (1), (2) and (3).

(4) Residual solvent Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum not exceeding 0.7 kPa, 100°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g, platinum crucible).

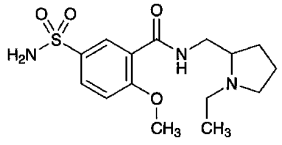
Assay Weigh accurately about 0.3 g of Sulindac, previously dried, dissolve in 50 mL of methanol and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 35.64 mg of $C_{20}H_{17}FO_3S$

Containers and storage Containers—Tight containers.

Sulpiride

スルピリド



$C_{15}H_{23}N_3O_4S$: 341.43
N-(1-Ethylpyrolidin-2-ylmethyl)-2-methoxy-5-sulfamoylbenzamide
[*15676-16-1*]

Sulpiride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{23}N_3O_4S$.

Description Sulpiride is a white, crystalline powder.

It is freely soluble in acetic acid (100) and in dilute acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is soluble in 0.05 mol/L sulfuric acid TS.

A solution of Sulpiride in methanol (1 in 100) shows no optical rotation.

Melting point: about 178°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Sulpiride in 0.05 mol/L sulfuric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sulpiride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity of solution—Dissolve 2.0 g of Sulpiride in 7 mL of dilute acetic acid, and add water to make 20 mL: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank: the absorbance at a wavelength of 450 nm does not exceed 0.020.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Sulpiride as directed under 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 50 mg of Sulpiride in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, accurately measured, with methanol to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with 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 ⟨*2.03*⟩. Spot 20 μL each of the sample 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-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution. When the plate is exposed to iodine vapor for 30 minutes, the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Sulpiride, previously dried and accurately weighed, in 80 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from violet through blue to bluish green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.14 mg of $C_{15}H_{23}N_3O_4S$

Containers and storage Containers—Well-closed containers.

Sulpiride Capsules

スルピリドカプセル

Sulpiride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Capsules, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the

Content uniformity test.

To 1 capsule of Sulpiride Capsules add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride ($C_{15}H_{23}N_3O_4S$), and filter the solution. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of sulpiride for assay

Dissolution Being specified separately.

Assay Cut the capsule of not less than 20 Sulpiride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride ($C_{15}H_{23}N_3O_4S$), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 m/L sulfuric acid TS 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 sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of sulpiride for assay

Containers and storage Containers—Tight containers.

Sulpiride Tablets

スルピリド錠

Sulpiride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Tablets, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sulpiride Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride ($C_{15}H_{23}N_3O_4S$), and filter the solution. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of sulpiride for assay

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg tablet in 30 minutes is not less than 80%, that of a 100-mg tablet in 45 minutes is not less than 75%, and that of a 200-mg tablet in 45 minutes is not less than 70%.

Start the test with 1 tablet of Sulpiride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 dissolution medium to make exactly V' mL so that each mL contains about 56 μg of sulpiride ($C_{15}H_{23}N_3O_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 291 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of sulpiride for assay
C: Labeled amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Sulpiride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride ($C_{15}H_{23}N_3O_4S$), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mL sulfuric acid TS 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 sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

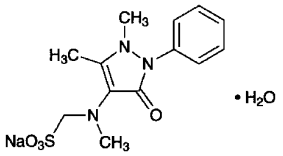
Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of sulpiride for assay

Containers and storage Containers—Tight containers.

Sulpyrine Hydrate

スルピリン水和物



$C_{13}H_{16}N_3NaO_4S.H_2O$: 351.35
Monosodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)(methyl)amino]methanesulfonate monohydrate
[*5907-38-0*]

Sulpyrine Hydrate contains not less than 98.5% of sulpyrine ($C_{13}H_{16}N_3NaO_4S$: 333.34), calculated on the dried basis.

Description Sulpyrine Hydrate occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

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

It is colored by light.

Identification **(1)** Add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS to 3 mL of a solution of Sulpyrine Hydrate (1 in 15): a deep blue color develops at first, but the color immediately turns red, then gradually changes to yellow.

(2) Boil 5 mL of a solution of Sulpyrine Hydrate (1 in 25) with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling, the odor of formaldehyde is perceptible.

(3) A solution of Sulpyrine Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Clarity of solution, and acidity or alkalinity—Dissolve 1.0 g of Sulpyrine Hydrate in 10 mL of water: the solution is clear and neutral.

(2) Sulfate <*1.14*>—Dissolve 0.20 g of Sulpyrine Hydrate in 0.05 mol/L hydrochloric acid VS 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 and 0.05 mol/L hydrochloric acid VS to make 50 mL (not more than 0.120%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Sulpyrine 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) Merbuline—Transfer 0.10 g of Sulpyrine Hydrate with 2 mL of water and 1 mL of dilute sulfuric acid into a flask, cover with a funnel, and boil gently for 15 minutes. Cool, add 2 mL of a solution of sodium acetate trihydrate (1 in 2) and water to make 5 mL, shake this solution with 5 mL of benzaldehyde-saturated solution, and allow to stand for 5 minutes: the solution is clear.

(5) Chloroform-soluble substances—Mix, by frequent shaking, 1.0 g of Sulpyrine Hydrate and 10 mL of chloroform for 30 minutes. Collect the precipitate, wash with two 5-mL portions of chloroform, combine the washings with the filtrate, and evaporate on a water bath to dryness. Dry the residue at 105°C for 4 hours: the mass of the residue is not more than 5.0 mg.

Loss on drying <*2.41*> Not more than 6.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.25 g of Sulpyrine Hydrate, dissolve in 100 mL of diluted hydrochloric acid (1 in 20), previously cooled below 10°C. Titrate <*2.50*> immediately with 0.05 mol/L iodine VS while keeping the temperature between 5°C and 10°C, until the color of the solution remains blue upon shaking vigorously for 1 minute after the addition of 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 16.67 mg of $C_{13}H_{16}N_3NaO_4S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sulpyrine Injection

スルピリン注射液

Sulpyrine Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$: 351.35).

Method of preparation Prepare as directed under Injections, with Sulpyrine Hydrate.

Description Sulpyrine Injection is a clear, colorless or pale yellow liquid.

pH: 5.0 – 8.5

Identification **(1)** To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 3 mL, then add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS: a deep blue color develops at first, and the color immediately turns red and gradually changes to yellow.

(2) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 5 mL, and boil this solution with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling the odor of formaldehyde is perceptible.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 2 mL of Sulpyrine Injection, dilute with water to exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 50 mg of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$), and add water to make exactly 100

mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of sulpyrine for assay (previously determine the loss on drying <2.41> in the same conditions as Sulpyrine Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution into separate 25-mL volumetric flasks, add 5 mL of ethanol (95), 2 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 250) and 2 mL of acetic acid (100) to each of these solutions, shake well, allow to stand for 15 minutes, and add water to exactly 25 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 2 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 510 nm.

Amount (mg) of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$) in 1 mL of Sulpyrine Injection
$= M_S \times A_T/A_S \times 50/V \times 1.054$

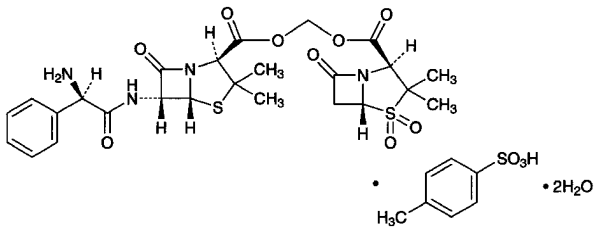
M_S: Amount (mg) of sulpyrine for assay, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and under nitrogen atmosphere.

Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物



$C_{25}H_{30}N_4O_9S_2.C_7H_8O_3S.2H_2O$: 802.89

(2*S*,5*R*)-(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-ylcarbonyloxy)methyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate mono-4-toluenesulfonate dihydrate [*83105-70-8*, anhydride]

Sultamicillin Tosilate Hydrate contains not less than 698 μg (potency) and not more than 800 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of residual solvent. The potency of Sultamicillin Tosilate Hydrate is expressed as mass (potency) of sultamicillin ($C_{25}H_{30}N_4O_9S_2$: 594.66).

Description Sultamicillin Tosilate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification Determine the infrared absorption spectrum of Sultamicillin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sultamicillin Tosilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +173 – +187° (0.5 g calculated on the anhydrous bases, a mixture of water and acetonitrile (3:2), 25 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sultamicillin Tosilate Hydrate, according to Method 3, and perform the test (not more than 2 ppm).

(3) Ampicillin—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 6 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 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of the peak of ampicillin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 80 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 14 minutes.

System suitability—

System performance: Dissolve 12 mg of Ampicillin RS, 4 mg of Sulbactam RS and 4 mg of *p*-toluenesulfonic acid monohydrate in 1000 mL of the mobile phase. When the procedure is run with 25 μL of this solution under the above operating conditions, sulbactam, *p*-toluenesulfonic acid and ampicillin 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 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ampicillin is not more than 2.0%.

(4) Sulbactam—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Sulbactam RS, equivalent to about 20 mg (potency), dissolve in 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 exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following con-

ditions, and determine the area of the peak of sulbactam by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3).

System suitability—

Proceed as directed in the system suitability in the Purity (3).

(5) Penicilloic acids—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, in a 100-mL flask with stopper. Add exactly 5 mL of 0.005 mol/L iodine VS, and allow to stand the stoppered flask for 5 minutes. Titrate ⟨*2.50*⟩ with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of penicilloic acid ($C_{25}H_{34}N_4O_{11}S_2$: 630.70) by using the following equation: it is not more than 3.0%.

$$\text{Each mL of 0.005 mol/L sodium thiosulfate VS} = 0.2585 \text{ mg of } C_{25}H_{34}N_4O_{11}S_2$$

(6) Residual solvent ⟨*2.46*⟩—Weigh accurately about 0.1 g of Sultamicillin Tosilate Hydrate, dissolve in 2 mL of methanol, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of ethyl acetate, and mix with water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of ethyl acetate of these solutions. Calculate the amount of ethyl acetate by the following equation: not more than 2.0%.

$$\text{Amount (\%) of ethyl acetate} = M_S/M_T \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of ethyl acetate
M_T: Amount (mg) of the sample

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 μm, 300 – 400 m^2/g) (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 155°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate 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, the number of theoretical plates and the symmetry factor of the peak of ethyl acetate are not less than 500 steps and not more than 1.5, respectively.

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 areas of ethyl acetate is not more than 5%.

Water ⟨*2.48*⟩ 4.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Perform the procedure rapidly. Weigh accurately an amount of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sultamicillin to that of the internal standard of each solution.

$$\text{Amount [}\mu\text{g (potency)] of sultamicillin } (C_{25}H_{30}N_4O_9S_2) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Sultamicillin Tosilate RS

Internal standard solution—A solution of isopropyl-4-aminobenzoate in the mobile phase (1 in 2500).

Operating conditions—

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

Column: A stainless steel column 3.9 mm in inside 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 35°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogenphosphate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 400 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of sultamicillin is about 4 minutes.

System suitability—

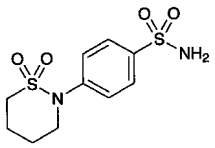
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, *p*-toluenesulfonic acid, sultamicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

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

Containers and storage Containers—Tight containers.

Sultiame

スルチアム



$C_{10}H_{14}N_2O_4S_2$: 290.36
4-(3,4,5,6-Tetrahydro-2*H*-1,2-thiazin-2-yl)benzenesulfonamide *S*,*S*-dioxide
[*61-56-3*]

Sultiame, when dried, contains not less than 98.5% of $C_{10}H_{14}N_2O_4S_2$.

Description Sultiame occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in *n*-butylamine, slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 0.02 g of Sultiame in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. To this solution add 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(2) Mix 0.1 g of Sultiame with 0.5 g of sodium carbonate decahydrate, and melt carefully: the gas evolved changes moistened red litmus paper to blue. After cooling, crush the fused substance with a glass rod, stir with 10 mL of water, and filter. To 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 formed.

(3) Determine the absorption spectrum of a solution of Sultiame in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 185 – 188°C

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 2 mL of acetic acid (100) and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent 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 as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 8 mL of sodium hydroxide TS, 0.8 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Sulfate <*1.14*>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 8 mL of dilute hydrochloric acid and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent 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 as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 8 mL of sodium hydroxide TS, 4.2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Sultiame 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 <*1.11*>—Prepare the test solution with 1.0 g of Sultiame according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sultiame in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of sulfanilamide in methanol to make exactly 100 mL. Pipet 10 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 <*2.03*>. Spot 20 μL each of the sample 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, methanol and ammonia solution (28) (30:8:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

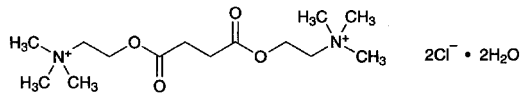
Assay Weigh accurately about 0.8 g of Sultiame, previously dried, dissolve in 70 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.2 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS
= 58.07 mg of $C_{10}H_{14}N_2O_4S_2$

Containers and storage Containers—Well-closed containers.

Suxamethonium Chloride Hydrate

スキサメトニウム塩化物水和物



$C_{14}H_{30}Cl_2N_2O_4.2H_2O$: 397.34
2,2′-Succinyldioxybis(*N*,*N*,*N*-trimethylethylaminium) dichloride dihydrate
[*6101-15-1*]

Suxamethonium Chloride Hydrate contains not less than 98.0% of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31), calculated on the anhydrous basis.

Description Suxamethonium Chloride Hydrate occurs as a

white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** Determine the infrared absorption spectrum of Suxamethonium Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Suxamethonium Chloride Hydrate (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ The pH of a solution of Suxamethonium Chloride Hydrate (1 in 100) is between 4.0 and 5.0.

Melting point ⟨*2.60*⟩ 159 – 164°C (hydrate form).

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

(2) Related substances—Dissolve 0.25 g of Suxamethonium Chloride Hydrate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 ⟨*2.03*⟩. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, n-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate, and allow to stand for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ 8.0 – 10.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Suxamethonium Chloride Hydrate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid VS
= 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers—Tight containers.

Suxamethonium Chloride for Injection

注射用スキサメトニウム塩化物

Suxamethonium Chloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31).

The concentration of Suxamethonium Chloride for Injection should be stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

Method of preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride for Injection occurs as a white, crystalline powder or mass.

Identification Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium 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°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and standard solution are blue-purple in color and have similar *R*f value.

pH ⟨*2.54*⟩ The pH of a solution of Suxamethonium Chloride for Injection (1 in 100) is between 4.0 and 5.0.

Purity Related substances—Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.25 g of Suxamethonium Chloride Hydrate according to the labeled amount, and proceed as directed in the Purity (2) under Suxamethonium Chloride Hydrate.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.5 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the contents of not less than 10 preparations of Suxamethonium Chloride for Injection. Weigh accurately about 0.5 g of the contents, and proceed as directed in the Assay under Suxamethonium Chloride Hydrate.

Each mL of 0.1 mol/L perchloric acid VS
= 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers—Hermetic containers.

Suxamethonium Chloride Injection

スキサメトニウム塩化物注射液

Suxamethonium Chloride Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31).

The concentration of Suxamethonium Chloride Injection should be stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

Method of preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride Injection is a clear, colorless liquid.

Identification Take a volume of Suxamethonium Chloride Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-later chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium 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°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and the standard solution are blue-purple in color and have similar *R*f value.

pH ⟨*2.54*⟩ 3.0 – 5.0

Purity Hydrolysis products—Perform the preliminary neutralization with 0.1 mol/L sodium hydroxide VS in the Assay: not more than 0.7 mL of 0.1 mol/L sodium hydroxide VS is required for each 200 mg of Suxamethonium Chloride ($C_{14}H_{30}Cl_2N_2O_4$) taken.

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.0 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requiremeut.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer to a separator an accurately measured volume of Suxamethonium Chloride Injection, equivalent to about 0.2 g of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$), add 30 mL of freshly boiled and cooled water, and wash the solution with five 20-mL portions of diethyl ether. Combine the diethyl ether washings, and extract the combined diethyl ether layer with two 10-mL portions of freshly boiled and cooled water. Wash the combined water extracts with two 10-mL portions of diethyl ether. Combine the solution and the water extracts, add 2 drops of bromothymol blue TS, and neutralize with 0.1 mol/L sodium hydroxide VS. Add accurately measured 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 40 minutes under a reflux condenser, and cool. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Transfer 50 mL of the freshly boiled and cooled water to a flask, add 2 drops of bromothymol blue TS, neutralize the solution with 0.1 mol/L sodium hydroxide VS, and perform a blank determination.

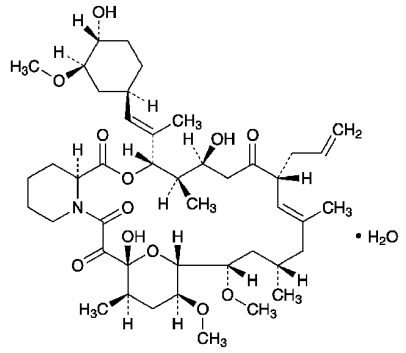
Each mL of 0.1 mol/L sodium hydroxide VS
= 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers—Hermetic containers.
Storage—Not exceeding 5°C, and avoid freezing.

Expiration date 12 months after preparation.

Tacrolimus Hydrate

タクロリムス水和物



$C_{44}H_{69}NO_{12}.H_2O$: 822.03

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-Dihydroxy-3-{(1*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-15,19-epoxy-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate

[*109581-93-3*]

Tacrolimus Hydrate contains not less than 98.0% and not more than 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$: 804.02), calculated on the anhydrous basis.

Description Tacrolimus Hydrate occurs as a white crystal or crystalline powder.

It is very soluble in methanol and in ethanol (99.5), freely soluble in *N*,*N*-dimethylformamide and in ethanol (95), and practically insoluble in water.

Identification **(1)** Dissolve 5 mg of Tacrolimus Hydrate in 1 mL of ethanol (95), add 1 mL of 1,3-dinitrobenzene TS and 1 mL of sodium hydroxide TS, and shake: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Tacrolimus Hydrate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Tacrolimus RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: −112 – −117° (0.2 g calculated on the anhydrous basis, *N*,*N*-dimethylformamide, 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Tacrolimus 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—Being specified separately.

(3) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ 1.9 – 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Isomer Being specified separately.

Assay Weigh accurately about 25 mg each of Tacrolimus Hydrate and Tacrolimus RS (separately determine the water ⟨*2.48*⟩ in the same manner as Tacrolimus Hydrate) and dissolve each in 15 mL of ethanol (99.5), to each add exactly 10 mL of the internal standard solution, add 25 mL of water, allow to stand for 6 hours, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tacrolimus to that of the internal standard.

$$\text{Amount (mg) of tacrolimus } (C_{44}H_{69}NO_{12}) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Tacrolimus RS, calculated on the anhydrous basis

Internal standard solution—A solution of heptyl parahydroxybenzoate in ethanol (99.5) (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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 50°C.

Mobile phase: A mixture of water, 2-propanol for liquid chromatography and tetrahydrofuran for liquid chromatography (5:2:2).

Flow rate: Adjust the flow rate so that the retention time of tacrolimus 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, tacrolimus and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

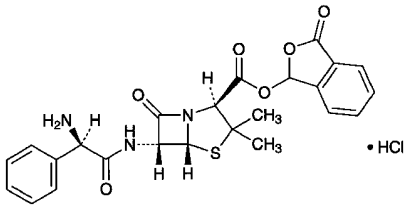
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tacrolimus to that of internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Talampicillin Hydrochloride

Ampicillinphthalidyl Hydrochloride

タランピシリン塩酸塩



$C_{24}H_{23}N_3O_6S.HCl$: 517.98

3-Oxo-1,3-dihydroisobenzofuran-1-yl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride

[*47747-56-8*]

Talampicillin Hydrochloride is the hydrochloride of ampicillin phthalidyl ester.

It contains not less than 600 μg (potency) and not more than 700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Talampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Talampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

Identification **(1)** To 1 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of sodium hydroxide TS, mix, allow to stand for 5 minutes, and add 2 mL of dilute sulfuric acid and 2 to 3 drops of 2,4-dinitrophenylhydrazine TS: an orange-yellow precipitate is formed.

(2) Determine the infrared absorption spectrum of Talampicillin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Talampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Talampicillin Hydrochloride (1 in 300) add 1 mL of dilute nitric acid, and add silver nitrate TS: a white precipitate is formed.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +151 – +171° (0.2 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Talampicillin 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Talampicillin Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL, 2 mL

and 3 mL of the sample solution, add ethanol (99.5) to each to make exactly 100 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, ethyl acetate, water and ethanol (95) (4:4:2:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (99.5) (1 in 500) on the plate, and heat at 110°C for 5 minutes: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution (3), and the total of the amount of each spot other than the principal spot from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (1), (2) and (3), is not more than 5%.

(4) 2-Formylbenzoic acid—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-formylbenzoic acid in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) 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 <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and acetic acid (100) (4:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of 2,4-dinitrophenylhydrazine in diluted sulfuric acid (6 in 25) (1 in 500): the spot of 2-formylbenzoic acid obtained from the sample solution is not more intense than that obtained from the standard solution.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Talampicillin Hydrochloride and Talampicillin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution. The standard solution should be prepared before use. Pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS, and allow them to stand for exactly 15 minutes. Add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, allow them to stand for exactly 15 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared. If necessary, add 0.2 to 0.5 mL of starch TS. Separately, pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add exactly 10 mL of 0.005 mol/L iodine VS, titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared, and make any necessary correction. For this titration, add 0.2 to 0.5 mL of starch TS, if necessary. Calculate the amount (mL) of 0.005 mol/L iodine VS, V_T and V_S, consumed by the sample solution and the standard solution, respectively.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) $= M_S \times V_T/V_S \times 1000$

M_S: Amount [mg (potency)] of Talampicillin Hydrochloride RS

Containers and storage Containers—Tight containers.

Talc

タルク

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Talc is a powdered, selected, natural, hydrated magnesium silicate. Pure talc is $Mg_3Si_4O_{10}(OH)_2$: 379.27. It may contain related mineral substances consisting chiefly of chlorite (hydrous magnesium aluminum silicate), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium magnesium carbonate).

It contains no asbestos.

It contains not less than 17.0% and not more than 19.5% of magnesium (Mg: 24.31).

◆**Description** Talc occurs as a white to grayish white, fine, crystalline powder.

It is unctuous, and adheres readily to the skin.

It is practically insoluble in water and in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Talc as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3680 cm^{-1}, 1018 cm^{-1} and 669 cm^{-1}.

Purity **(1)** Acidity or alkalinity—To 2.5 g of Talc add 50 mL of freshly boiled and cooled water, and heat under a reflux condenser. Filter the liquid by suction, add 0.1 mL of bromothymol blue-sodium hydroxide-ethanol TS to 10 mL of the filtrate, and add 0.01 mol/L hydrochloric acid VS until the color of the solution changes: the necessary volume of the VS is not more than 0.4 mL. Separately, to 10 mL of the filtrate add 0.1 mL of phenolphthalein TS, and add 0.01 mol/L sodium hydroxide VS until the color of the solution changes to light red: the necessary volume of the VS is not more than 0.3 mL.

◆**(2)** Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of the filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800 ± 25°C: the amount of the residue is not more than 2.0%.◆

◆**(3)** Water-soluble substances—To 10.0 g of Talc add 50 mL of water, weigh the mass, and boil for 30 minutes, supplying water lost by evaporation. Cool, add water to restore the original mass, and filter. Centrifuge, if necessary, until the filtrate becomes clear. Evaporate 20 mL of the filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 4.0 mg.◆

(4) Iron—Weigh accurately about 10 g of Talc, add 50 mL of 0.5 mol/L hydrochloric acid TS gently while stirring, and heat under a reflux condenser on a water bath for 30

minutes. After cooling, transfer the content to a beaker, and allow to settle the insoluble matter. Filter the supernatant liquid through a filter paper for quantitative analysis (No. 5B), leaving the precipitate in the beaker as much as possible, wash the remaining precipitate in the beaker with three 10-mL portions of hot water, and also wash the filter paper with 15 mL of hot water, and combine the washings and the filtrate. After cooling, add water to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 2.5 mL of the stock solution, add 50 mL of 0.5 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 50 mL each of 0.5 mol/L hydrochloric acid TS add exactly 2 mL, 2.5 mL, 3 mL and 4 mL of Standard Iron Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of iron from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.25%.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Iron hollow-cathode lamp.
Wavelength: 248.3 nm.

(5) Aluminum—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of cesium chloride TS and 10 mL of hydrochloric acid, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of cesium chloride TS add exactly 5 mL, 10 mL, 15 mL and 20 mL of Standard Aluminum Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of aluminum from the calibration curve prepared from the absorbances of the standard solutions: not more than 2.0%.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.
Lamp: Aluminum hollow-cathode lamp.
Wavelength: 309.3 nm.

(6) Lead—Use the sample stock solution obtained in (4) as the sample solution. Separately, to 50 mL of 0.5 mol/L hydrochloric acid TS add exactly 5 mL, 7.5 mL, 10 mL and 12.5 mL of Standard Lead Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of lead from the calibration curve prepared from the absorbances of the standard solutions: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 217.0 nm.

(7) Calcium—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 1 mL, 2 mL, 3 mL and 5 mL of Standard Calcium Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of calcium from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.9%.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

◆**(8)** Arsenic <*1.11*>—To 0.5 g of Talc add 5 mL of dilute sulfuric acid, and heat gently to boiling with shaking. Cool immediately, filter, and wash the residue with 5 mL of dilute sulfuric acid, then with 10 mL of water. Combine the filtrate and the washings, evaporate to 5 mL on a water bath, and perform the test with this solution as the test solution (not more than 4 ppm).◆

Loss on ignition <*2.43*> Not more than 7.0% (1 g, 1050 – 1100°C, constant mass).

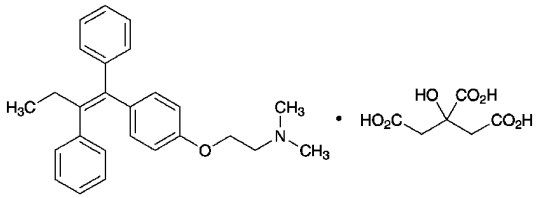
Assay Weigh accurately about 0.5 g of Talc in a polytetrafluoroethylene dish, add 5 mL of hydrochloric acid, 5 mL of nitric acid and 5 mL of perchloric acid, then add 35 mL of hydrofluoric acid while mixing gently, and evaporate to dryness on a hot plate by heating gradually. Add 5 mL of hydrochloric acid to the residue, cover the dish with a watch glass, and heat to boil. After cooling, transfer the content to a volumetric flask while washing the watch glass and dish with water, further wash the dish with water, transfer the washings to the flask, then add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 0.5 mL of the sample stock solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 2.5 mL, 3 mL, 4 mL and 5 mL of Standard Magnesium Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of magnesium from the calibration curve prepared from the absorbances of the standard solutions.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Magnesium hollow-cathode lamp.
Wavelength: 285.2 nm.

◆**Containers and storage** Containers—Well-closed containers.◆

Tamoxifen Citrate

タモキシフェンクエン酸塩



$C_{26}H_{29}NO.C_6H_8O_7$: 563.64
2-{4-[(1*Z*)-1,2-Diphenylbut-1-en-1-yl]phenoxy}-*N*,*N*-dimethylethylamine monocitrate
[*54965-24-1*]

Tamoxifen Citrate, when dried, contains not less than 99.0% and not more than 101.0% of $C_{26}H_{29}NO.C_6H_8O_7$.

Description Tamoxifen Citrate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Tamoxifen Citrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamoxifen Citrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tamoxifen Citrate (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for citrate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Tamoxifen 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).

(2) Related substances—Conduct this procedure rapidly, using light-resistant vessels. Dissolve 15 mg of Tamoxifen Citrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than tamoxifen obtained from the sample solution is not larger than 3/10 times the peak area of tamoxifen from the standard solution, and the total area of the peaks other than the peak of tamoxifen from the sample solution is not larger than 4/5 times the peak area of tamoxifen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside 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 4.8 g of *N*,*N*-dimethyl-*n*-octylamine in 1000 mL of water. Separately, dissolve 0.9 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. Mix these solutions, and adjust to pH 3.0 with phosphoric acid. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tamoxifen is about 21 minutes.

Time span of measurement: About 2.5 times as long as the retention time of tamoxifen, beginning after the solvent peak.

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 tamoxifen 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 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tamoxifen are not less than 5000 and not more than 1.5, respectively.

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

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tamoxifen Citrate, previously dried, dissolve in 150 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

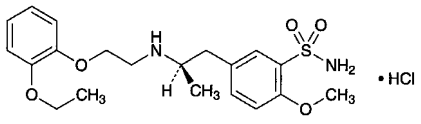
Each mL of 0.1 mol/L perchloric acid VS
= 56.36 mg of $C_{26}H_{29}NO.C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Tamsulosin Hydrochloride

タムスロシン塩酸塩



$C_{20}H_{28}N_2O_5S.HCl$: 444.97

5-{(2*R*)-2-[2-(2-Ethoxyphenoxy)ethylamino]propyl}-2-methoxybenzenesulfonamide monohydrochloride

[*106463-17-6*]

Tamsulosin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{20}H_{28}N_2O_5S.HCl$.

Description Tamsulosin Hydrochloride occurs as white crystals.

It is freely soluble in formic acid, sparingly soluble in water, slightly soluble in acetic acid (100), and very slightly soluble in ethanol (99.5).

Melting point: about 230°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Tamsulosin Hydrochloride (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamsulosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of an ice cooled solution of Tamsulosin Hydrochloride (3 in 400) add 3 mL of dilute nitric acid, shake well, allow to stand at room temperature for 30 minutes, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -17.5 – $-20.5°$ (after drying, 0.15 g, water, warming, after cooling, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Tamsulosin 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).

(2) Related substances—

(i) Dissolve 50 mg of Tamsulosin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2.5 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 6 minutes.

Time span of measurement: Until tamsulosin is eluted, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μL of this solution is equivalent to 1.4 to 2.6% of that from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Tamsulosin Hydrochloride and 10 mg of propyl parahydroxybenzoate in 20 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, tamsulosin and propyl parahydroxybenzoate are eluted in this order with the resoluton between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

(ii) Perform the test with 10 μL each of the sample solution and standard solution which are obtained in above (i) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (2) (i).

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 2.5 minutes.

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

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase used in the Purity (2) (i) to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μL of this solution is equivalent to 1.4 to 2.6% of that from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Purity (2) (i).

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Tamsulosin Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 75 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and immediately titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.50 mg of $C_{20}H_{28}N_2O_5S.HCl$

Containers and storage Containers—Well-closed containers.

Tamsulosin Hydrochloride Extended-release Tablets

タムスロシン塩酸塩徐放錠

Tamsulosin Hydrochloride Extended-release Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$: 444.97).

Method of preparation Prepare as directed under Tablets, with Tamsulosin Hydrochloride.

Identification To an amount of powdered Tamsulosin Hydrochloride Extended-release Tablets, equivalent to 1 mg of Tamsulosin Hydrochloride according to the labeled amount, add about 5 g of porcelain balls with about 5 mm in diameter, add 20 mL of 0.2 mol/L sodium hydroxide TS, warm at 50°C for 10 minutes, and shake vigorously for 15 minutes. Then, add 7 mL of acetonitrile, shake slightly, and centrifuge. Take the supernatant liquid, add 2.5 g of sodium chloride and 5 mL of ethyl acetate, shake vigorously for 5 minutes, and centrifuge. Take the supernatant liquid, evaporate to dryness at 50°C in a water bath, dissolve the residue with 20 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 222 nm and 226 nm, and between 278 nm and 282 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tamsulosin Hydrochloride Extended-release Tablets add about 5 g of porcelain balls with about 5 mm in diameter and 5 mL of water, and shake to disintegrate the tablet. Add 20 mL of a solution of sodium hydroxide (1 in 500), warm at 50°C for 10 minutes, shake vigorously for 30 minutes, and add 10 mL of acetonitrile and 5 mL of 0.2 mol/L hydrochloric acid TS. To this solution add exactly 5 mL of the internal standard solution for every 0.1 mg of tamsulosin hydrochloride, add the mobile phase to make 50 mL, shake slightly, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To V mL of the filtrate add the mobile phase to make V' mL so that each mL contains about 2 μg of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$)
$= M_S \times Q_T/Q_S \times V'/V \times 1/100$

M_S: Amount (mg) of tamsulosin hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tamsulosin Hydrochloride Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 mg of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$), add about 5 g of porcelain balls with about 5 mm in diameter and 5 mL of water, shake, then add 20 mL of a solution of sodium hydroxide (1 in 500), warm at 50°C for 10 minutes, and shake vigorously for 30 minutes. To this solution add 10 mL of acetonitrile, 5 mL of 0.2 mol/L hydrochloric acid TS and exactly 5 mL of the internal standard solution, then add 5 mL of the mobile phase, shake slightly, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of tamsulosin hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tamsulosin to that of the internal standard.

Amount (mg) of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$)
$= M_S \times Q_T/Q_S \times 1/100$

M_S: Amount (mg) of tamsulosin hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust to pH 2.0 with sodium hydroxide TS, and add water to make 1000

mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin 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, the internal standard and tamsulosin 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tamsulosin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tannic Acid

タンニン酸

Tannic Acid is the tannin usually obtained from nutgalls or rhusgalls.

Description Tannic Acid occurs as a yellowish white to light brown, amorphous powder, glistening leaflets, or spongy masses. It is odorless or has a faint, characteristic odor, and has a strongly astringent taste.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** To 5 mL of a solution of Tannic Acid (1 in 400) add 2 drops of iron (III) chloride TS: a blue-black color develops. Allow the solution to stand: a blue-black precipitate is produced.

(2) To 5 mL of a solution of Tannic Acid (1 in 20) add 1 drop each of albumin TS, gelatin TS, or 1 mL of starch TS: a precipitate is produced in each solution.

Purity **(1)** Gum, dextrin and sucrose—Dissolve 3.0 g of Tannic Acid in 15 mL of boiling water: the solution is clear or slightly turbid. Cool, and filter the solution. To 5 mL of the filtrate add 5 mL of ethanol (95): no turbidity is produced. Add further 3 mL of diethyl ether to this solution: no turbidity is produced.

(2) Resinous substances—To 5 mL of the filtrate obtained in (1) add 10 mL of water: no turbidity is produced.

Loss on drying ⟨*2.41*⟩ Not more than 12.0% (1 g, 105°C, 2 hours).

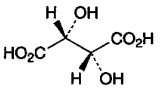
Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (0.5 g).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tartaric Acid

酒石酸



$C_4H_6O_6$: 150.09

(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid

[*87-69-4*]

Tartaric Acid, when dried, contains not less than 99.7% of $C_4H_6O_6$.

Description Tartaric Acid occurs as colorless crystals or a white, crystalline powder. It is odorless, and has a strong acid taste.

It is very soluble in water, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

A solution of Tartaric Acid (1 in 10) is dextrorotatory.

Identification **(1)** Ignite Tartaric Acid gradually: it decomposes and an odor of burning sugar is perceptible.

(2) A solution of Tartaric Acid (1 in 10) changes blue litmus paper to red, and responds to the Qualitative Tests ⟨*1.09*⟩ for tartrate.

Purity **(1)** Sulfate ⟨*1.14*⟩—Perform the test with 0.5 g of Tartaric Acid. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Oxalate—Dissolve 1.0 g of Tartaric Acid in 10 mL of water, and add 2 mL of calcium chloride TS: no turbidity is produced.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Tartaric Acid 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) Calcium—Neutralize a solution of 1.0 g of Tartaric Acid in 10 mL of water with ammonia TS, and add 1 mL of ammonium oxalate TS: no turbidity is produced.

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Tartaric Acid according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (3 g, silica gel, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (1 g).

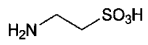
Assay Weigh accurately about 1.5 g of Tartaric Acid, previously dried, dissolve in 40 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 75.05 mg of $C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Taurine

タウリン



$C_2H_7NO_3S$: 125.15
2-Aminoethanesulfonic acid
[*107-35-7*]

Taurine, when dried, contains not less than 99.0% and not more than 101.0% of $C_2H_7NO_3S$.

Description Taurine occurs as colorless or white crystals, or a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 1.0 g of Taurine in 20 mL of freshly boiled and cooled water is between 4.1 and 5.6.

Identification Determine the infrared absorption spectrum of Taurine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Taurine in 20 mL of water is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 1.0 g of Taurine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <*1.14*>—Perform the test with 2.0 g of Taurine. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of Taurine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Taurine 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).

(6) Iron <*1.10*>—Prepare the test solution with 2.0 g of Taurine according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 1.0 g of Taurine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ethanol (99.5), 1-butanol and acetic acid (100) (150:150:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot other than the principle spot with the sample solution is not more than one spot, and it is not more intense than the spot with the standard solution.

Loss on drying <*2.41*> Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

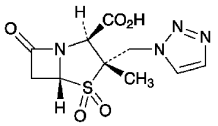
Assay Weigh accurately about 0.2 g of Taurine, previously dried, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.52 mg of $C_2H_7NO_3S$

Containers and storage Containers—Well-closed containers.

Tazobactam

タゾバクタム



$C_{10}H_{12}N_4O_5S$: 300.29
(2*S*,3*S*,5*R*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide
[*89786-04-9*]

Tazobactam contains not less than 980 μg and not more than 1020 μg (potency) per 1 mg, calculated on the anhydrous basis. The potency of Tazobactam is expressed as mass (potency) of $C_{10}H_{12}N_4O_5S$.

Description Tazobactam occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in dimethylsulfoxide and in *N*,*N*-dimethylformamide, and slightly soluble in water, in methanol and in ethanol (99.5).

It dissolves in a solution of sodium hydrogen carbonate (3 in 100).

Identification **(1)** Determine the infrared absorption spectrum of Tazobactam as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tazobactam RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Tazobactam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 35) as directed under the Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.3 ppm, and double signals, B and C, at around δ 7.8 ppm and at around δ 8.1 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +162 – +167° (1 g calculated on the anhydrous basis, *N*,*N*-dimethylformamide, 100 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Tazobactam in 10 mL of sodium hydrogen carbonate (3 in 100): the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 420 nm is not more than 0.14.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tazobactam 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—This operation must be performed quickly. Dissolve 50 mg of Tazobactam in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). 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 (2). Perform the test with exactly 50 μL each of the sample solution, the standard solutions (1) and (2) as directed under the Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.17 with respect to tazobactam, obtained from the sample solution is not larger than 4/5 times the peak area of tazobactam from the standard solution (1), the area of the peak other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect to tazobactam from the sample solution is not larger than the peak area of tazobactam from the standard solution (2), and the total area of the peaks other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect of tazobactam from the sample solution is not larger than 2 times the peak area of tazobactam from the standard solution (2).

Operating conditions—

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

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution (1), and add the mobile phase to make exactly 20 mL. Confirm that the peak area of tazobactam obtained from 50 μL of this solution is equivalent to 3 to 7% of that of tazobactam from 50 μL of the standard (1).

System performance: When the procedure is run with 50 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 2000 and 0.8 – 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution (1) under the above operating conditions, the relative standard deviations of the peak area of tazobactam is not more than 1.0%.

(4) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 0.5% (1 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).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Bacterial endotoxins <*4.01*> Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Tazobactam and Tazobactam RS, equivalent to about 50 mg (potency), dissolve each in exactly 10 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of tazobactam to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of tazobactam } (C_{10}H_{12}N_4O_5S) = M_S \times (Q_T/Q_S) \times 1000$$

M_S: Amount [mg (potency)] of Tazobactam RS

Internal standard solution—A solution of phenylalanine (1 in 400).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 1.32 g of diammonium hydrogen phosphate in 750 mL of water, adjust the pH to 2.5 with phosphoric acid, add water to make 1000 mL, and add 25 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tazobactam 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 tazobactam 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tazobactam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Teceleukin (Genetical Recombination)

テセロイキン（遺伝子組換え）

$C_{698}H_{1127}N_{179}O_{204}S_8$: 15547.01
[*136279-32-8*]

The desired product of Teceleukin (Genetical Recombination) is a protein consisting of 134 amino acid residues manufactured by *E. coli* through expression of human interleukin-2 cDNA.

It is a solution and possesses a T-lymphocyte activating effect.

It contains potency between 7.7×10^6 and 1.54×10^7 units per mL, and not less than 7.7×10^6 units per mg of protein.

Description Teceleukin (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** Measure accurately an appropriate amount of Teceleukin (Genetical Recombination), add accurately to a concentration of 200 units per mL of culture medium for assay of teceleukin, and use this solution as the sample stock solution. Dilute reference anti-interleukin-2 antibody for teceleukin with culture medium for assay of teceleukin to a concentration of approximately 200 neutral units per mL and use this solution as the interleukin-2 neutral antibody solution. Accurately add an equivalent volume of the interleukin-2 neutral antibody solution to the sample stock solution, shake, and then leave for 1 hour in a 37°C incubator in air containing 5% carbon dioxide. This solution is the sample solution. Prepare a standard solution by accurately adding an equivalent volume of culture medium for assay of teceleukin to the sample stock solution, mixing, and then processing in the same way. Process the sample and standard solutions according to the assay method, determine their respective dilution coefficients, D_N and D_T, and then calculate the neutralization rate, which should be at least 90%, using the following formula.

$$\text{Neutralization rate (\%)} = (D_T - D_N)/D_T \times 100$$

However, please note if the mean values of the absorbance of the maximum uptake control solution and absorbance of the minimum uptake control solution do not fit the standard curve, the neutralization coefficient is to be determined within the following range.

$$\text{Neutralization coefficient (\%)} > (D_T - 2)/D_T \times 100$$

(2) Place a volume of Teceleukin (Genetical Recombination) corresponding to approximately 50 μg of protein into 2 test tubes for hydrolysis, evaporate to dryness under vacuum, and use one as the sample (1). To the other, add 50 μL of a mixture of formic acid and hydrogen peroxide (30) (9:1) that has been left at room temperature for one hour, cool for 4 hours in ice, add 0.5 mL of water, and then evaporate to dryness under vacuum to give the sample (2). To 1.3 mL of methanesulfonic acid add 3.7 mL of water, mix well, add and dissolve 10 mg of 3-(2-aminoethyl)indole, to make a 4 mol/L methanesulfonic acid solution. Dissolve 39.2 g of trisodium citrate dihydrate, 33 mL of hydrochloric acid, 40 mL of thiodiglycol, and 4 mL of lauromacrogol solution (1 in 4) in 700 mL of water, adjust the pH to 2.2, add water to 1000 mL, add 100 μL of capric acid, and mix to make a sodium citrate solution for dilution. Add 50 μL of freshly prepared 4 mol/L methanesulfonic acid to the sample (1) and sample (2), cool to −70°C, and then deaerate under vacuum. Heat to 115°C ± 2°C for 24 hours after sealing these test tubes under reduced pressure. After cooling, unseal, add 50 μL of 4 mol/L sodium hydroxide TS followed by 0.4 mL of sodium citrate solution for dilution to make the sample solution (1) and sample solution (2). Separately, accurately measure 0.25 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate, and L-arginine hydrochloride as well as 0.125 mmol of L-cysteine and then dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. This is the amino acid standard stock solution. Accurately measure 1 mL of this solution, and add sodium citrate solution for dilution to make exactly 25 mL. This is solution A. Accurately weigh approximately 20 mg of L-tryptophan and dissolve in water to make exactly 1000 mL. This is solution B. Accurately measure 10 mL of both solution A and solution B, combine together, and add sodium citrate solution for dilution to make exactly 50 mL. This is the amino acid standard solution. Separately, accurately weigh approximately 17 mg of L-cysteic acid and dissolve in sodium citrate solution for dilution to make exactly 50 mL. Accurately measure 1 mL of this solution and add sodium citrate solution for dilution to make exactly 100 mL. This is the cysteic acid standard solution. Accurately measure 0.25 mL of the sample solution (1), the sample solution (2), amino acid standard solution, and the cysteic acid standard solution. When the test is conducted by Liquid Chromatography <*2.01*> under the following conditions, peaks for the 18 amino acids are observed in the chromatogram obtained from the sample solution (1). Also, measure the peak area of each amino acid in the sample solution (1) and the amino acid standard solution, and taking the molar number of alanine in the sample solution (1) as 5.0, determine the concentrations of aspartic acid, glutamic acid, proline, glycine, methionine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan, and arginine and then calculate the molar ratio for each amino acid. Also, measure the cysteic acid peak areas of the sample solution (2) and the cysteic acid standard solution, determine the concentration of cysteine, and, taking the molar number of alanine in the sample solution (2) as 5.0, calculate the molar ratio of cysteine. When determining the molar ratios of the respective amino acids, aspartic acid is 11.4 to 12.6, glutamic acid 17.1 to 18.9, proline 4.5 to 5.5, glycine 1.8 to 2.2, cysteine 2.7 to 3.3, methionine 4.5 to 5.5, leucine 20.9 to 23.1, tyrosine 2.7 to 3.3, phenylalanine 5.4 to 6.6, lysine 10.5 to 11.6, histidine 2.7 to 3.3, tryptophan 0.7 to 1.2, and arginine 3.6 to 4.4.

Operating conditions—

Detector: Visible absorption photometer [wavelengths: 440 nm (proline) and 570 nm (amino acids other than proline)]

Column: A stainless steel column with an inside diameter of 4 mm and length of 25 cm packed with a strongly acidic ion exchange resin for liquid chromatography consisting of polystyrene to which sulphonate group binds.

Column temperature: A constant temperature of about 50°C when the sample is injected. After a certain time, increase the temperature to a constant temperature of about 62°C.

Reaction temperature: A constant temperature of about 98°C.

Time for color formation: Approximately 2 minutes.

Mobile phase: After preparing mobile phases A, B, and C according to the following table, add 0.1 mL of capric acid to each.

	Mobile phase A	Mobile phase B	Mobile phase C
Citric acid monohydrate	18.70 g	10.50 g	7.10 g
Trisodium citrate dihydrate	7.74 g	14.71 g	26.67 g
Sodium chloride	7.07 g	2.92 g	54.35 g
Ethanol (99.5)	60 mL	—	—
Benzyl alcohol	—	—	10 mL
Thiodiglycol	5 mL	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount
pH	3.2	4.3	4.7
Total volume	1000 mL	1000 mL	1000 mL

Changing mobile phases and column temperature: When operating under the above conditions using 0.25 mL of amino acid standard solution, the amino acids will elute in the following order; aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, tryptophan, and arginine. Switchover to mobile phase A, mobile phase B, and mobile phase C, in sequence so that the resolution between the peaks of cystine and valine is 2.0 or more and that between ammonia and histidine is 1.5 or more. Also, increase the temperature after a constant length of time so that the resolution between the peaks of glutamic acid and proline is at least 2.0.

Reaction reagents: Dissolve 408 g of lithium acetate dihydrate in water, and add 100 mL of acetic acid (100) and water to make 1000 mL. To this solution add 1200 mL of dimethylsulfoxide and 800 mL of 2-methoxyethanol. This is solution (I). Separately, mix together 600 mL of dimethylsulfoxide and 400 mL of 2-methoxyethanol and then add 80 g of ninhydrin and 0.15 g of sodium borohydride. This is solution (II). After gassing 3000 mL of the solution (I) for 20 minutes with nitrogen, rapidly add 1000 mL of the solution (II) and then mix by gassing for 10 minutes with nitrogen.

Mobile phase flow rate: About 0.275 mL every minute.

Reaction reagent flow rate: About 0.3 mL every minute.

System suitability—

System performance: When 0.25 mL of the amino acid standard solution is run under the above conditions, the resolution between the peaks of threonine and serine is at least 1.5.

(3) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate, and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. After adjusting the pH to 8.0 using 1 mol/L hydrochloric acid TS, add water to make 100 mL. This is the molecular weight determination buffer solution. Accurately measure 20 μL of Teceleukin (Genetical Recombination), add exactly 20 μL of the molecular weight determination buffer solution and 2 μL of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μL of bromophenol blue solution (1 in 2000) and then shake. This is the sample solution. Separately, accurately measure 5 μL of molecular weight marker for teceleukin, and add exactly 50 μL of water, 55 μL of the molecular weight determination buffer solution, and 5 μL of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μL of bromophenol blue solution (1 in 2000), and shake well. This is the molecular weight standard solution. When conducting a test using SDS-polyacrylamide gel electrophoresis with 1 μL each of the sample solution and the molecular weight standard solution, the molecular weight of the main band is between the range of 14,000 and 16,000.

Operating conditions—

Equipment: Horizontal electrophoresis vessel equipped with a cooling unit, a device that accumulates load voltage over time, and a direct current power source device that controls the amperage, voltage, wattage.

Spotting of solutions: Solutions are spotted on concentrating gel of polyacrylamide gel sheets.

Electrophoresis conditions

Polyacrylamide gel sheet: Polyester sheet to which a polyacrylamide gel (width, about 43 mm, length, about 50 mm, and thickness, about 0.5 mm) is closely adhered. The polyacrylamide gel consists of a concentrating gel with a gel support concentration of 7.5% and a 3% degree of crosslinking and a separating gel with corresponding values of 20% and 2%. The gel contains pH 6.5 Tris-acetate buffer.

Buffer solution for electrode: Prepared by dissolving 35.83 g of tricine, 24.23 g of 2-amino-2-hydroxymethyl-1,3-propanediol, and 5.5 g of sodium lauryl sulfate in water to make 1000 mL.

Cooling temperature of gel support plate: 15°C.

Running conditions

Pre-electrophoresis and electrophoresis: The voltage, amperage, and wattage should not exceed 250 V, 10 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Immediately after adding sample: The voltage, amperage, and wattage should not exceed 250 V, 1 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Electrophoresis time

Before adding sample: Until value of load voltage integrated with respect to time reaches 60 V·h.

Immediately after adding sample: Until value of load voltage integrated with respect to time reaches 1 V·h.

Main electrophoresis: Until value of load voltage integrated with respect to time reaches 140 V·h.

Fixation and staining

Dissolve 25 g of anhydrous sodium carbonate anhydride and 0.8 mL of formaldehyde solution in water to make 1000 mL. This is the developing solution. After immersing

the polyacrylamide gel sheet in a mixture of ethanol (99.5), water and acetic acid (100) (5:4:1) for 2 minutes, immerse for 2 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (17:2:1). Change the mixture, immerse for another 4 minutes, immerse in water for 2 minutes to rinse the polyacrylamide gel sheet, and change the water to immerse for 2 minutes. This procedure is carried out with heating to 50°C. Next, while heating at 40°C, immerse for 10 to 15 minutes in diluted silver nitrate TS (1 in 7), warm to 30°C, and gently rinse the polyacrylamide gel sheet with water. While warming at 30°C, immerse the polyacrylamide gel sheet in freshly prepared developing solution. After obtaining adequate color formation, immerse the polyacrylamide gel sheet in diluted acetic acid (100) (1 in 20) to terminate the color formation.

Estimation of molecular weight

Plot graphs for each band obtained from the molecular weight standard solution, distance from the border of the concentrating gel and separating gel, and the logarithm of the molecular weight of proteins in each band. Calculate the molecular mass by reading the corresponding position of the major band obtained from the sample solution on the graph.

(4) The isoelectric point determined from the electrophoresis position is 7.4 to 7.9 when 3 μL of Teceleukin (Genetical Recombination) and 8 μL of isoelectric marker for teceleukin are tested by the polyacrylamide gel isoelectric method.

Operating conditions—

Equipment: Horizontal electrophoretic vessel with cooling unit and direct current power source that can perform constant wattage control.

Preparation of polyacrylamide gel: Dissolve 1.62 g of polyacrylamide and 50 mg of *N*,*N*′-methylenebisacrylamide in water to make 25 mL. Accurately measure 7.5 mL of this solution, 2 mL of a 10 mL solution prepared by adding water to 5 g of glycerin, and 0.64 mL of a pH 3 to pH 10 amphoteric electrolyte solution, and degas under reduced pressure while stirring thoroughly. Next, accurately measure 74 μL of freshly prepared ammonium peroxodisulfate solution (1 in 50), 3 μL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and 50 μL of freshly prepared riboflavin sodium phosphate solution (1 in 1000), stir well, immediately pour on a gel preparation plate (10 cm wide, 11 cm long, and 0.8 mm thick), and then expose to a fluorescent light source for 60 minutes to gelate.

Spotting

Add Teceleukin (Genetical Recombination) or isoelectric marker for teceleukin 30 minutes after starting electrophoresis to wells in gel plates to which plastic tape (3.5 mm wide, 3.5 mm long, 0.4 mm thick) has been applied in advance and that have undergone gelation.

Electrophoresis conditions

Cathode solution: Sodium hydroxide TS.

Anode solution: DL-aspartic acid solution (133 in 25,000).

Cooling temperature of gel support plate: 2 ± 1°C.

Running conditions: After starting the electrophoresis, a constant wattage of 10 W for 20 minutes and 20 W thereafter. However, the voltage should be 3000 V or less.

Running time: 120 to 140 minutes while blowing Nitrogen into the electrophoresis vessel.

Fixation and washing

Dissolve 28.75 g of trichloroacetic acid and 8.65 g of 5-sulfosalicylic acid dihydrate in 75 mL of methanol and 175 mL of water. Immerse the gel in this solution for 60 minutes to fix the protein to the gel. After fixation, immerse for 10 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Staining and decolorization

Dissolve 0.11 g of Coomassie brilliant blue G-250 in 25 mL of ethanol (99.5), and add 8 mL of acetic acid (100) and water to make 100 mL. This is the staining solution. Immerse the gel for 10 minutes while heating at 60°C in freshly filtered staining solution. After staining, decolorize by immersing in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Determination of isoelectric point

Plot the protein isoelectric points and the distance from the cathode of each band obtained from the isoelectric markers for teceleukin. Calculate the isoelectric point from the corresponding position of the major bands obtained from the sample solution.

pH ⟨*2.54*⟩ 2.7 – 3.5

Purity **(1)** Host cell-derived protein—Take an appropriate amount of Teceleukin (Genetical Recombination) and add an exact amount of diluted acetic acid (100) (1 in 350) to make a solution containing between 0.68 and 0.72 mg of protein in one mL. This is the sample stock solution. Dissolve 1.52 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 10.94 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 200 mL. Dissolve 0.5 g of bovine serum albumin in 25 mL of this solution. This is 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution. Accurately measure 0.5 mL of the sample stock solution, add exactly 30 μL of sodium carbonate TS, stir, and immediately add exactly 0.47 mL of the 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make the sample solution. Accurately measure 10 mL of dilute acetic acid (100) (1 in 350), add 0.6 mL of sodium carbonate TS, and then add 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make exactly 20 mL. This is the dilution solution. Add the E. coli protein stock solution to this dilution solution to make a solution containing 0.015 μg of E. coli protein in one mL. This is standard solution (1). Accurately dilute this solution serially two-fold with the dilution solution to make standard solutions (2) to (8) having different concentrations of E. coli protein. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4. This is the wash solution. Accurately measure 0.1 mL of the sample solution, standard solutions (1) to (8), and dilution solution as a blank standard solution and place each in 3 wells in solid phase plates (place dilution solution in 6 wells), cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 5 to 16 hours. Next, remove the solution from each well by aspiration, add 0.25 mL of the the wash solution, mix again by shaking in a horizontal direction, and then remove by aspiration. Repeat this procedure 2 more times by adding 0.25 mL of the wash solution to each well. Freshly dilute peroxidase marker antibody stock solution with 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS, add exactly 0.1 mL to each well, cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing

at a constant temperature of about 25°C for 16 to 24 hours. Next, remove the solution in the wells by aspiration, add 0.25 mL of the wash solution, mix by shaking in a horizontal direction, and then remove the solution by aspiration. Using 0.25 mL of the wash solution, repeat this procedure 2 more times for each well. To each well, accurately add 0.1 mL of teceleukin chromophore solution, stir gently, and then shield from light and leave standing for 30 minutes at a constant temperature of about 25°C. Add exactly 0.1 mL of diluted sulfuric acid (3 in 50) to each well and then mix by gently shaking horizontally. Measure the absorbances of these solutions A_{T2} and A_{S2} at 450 nm and A_{T1} and A_{S1} at 510 nm. Prepare a standard curve by plotting the values obtained from each standard solution ($A_{S2} - A_{S1}$) on a graph having the concentration of E. coli protein (ng/mL) in logarithmic scale on the horizontal axis and the absorbance values on the vertical axis. Match the values obtained from the sample solution ($A_{T2} - A_{T1}$) to the standard curve, determine the concentration A of E. coli protein in the sample solution, and take the mean. The amount of E. coli protein is not more than 5 ng when the amount of E. coli protein per mg of protein is determined using the following formula.

Amount (ng) of E. coli-derived protein per mg protein $= A/C$

C: Protein concentration (mg/mL) in sample solution

The test is valid if the E. coli protein concentration is 0.3 ng/mL or less when the concentration is obtained by fitting the absorbance value at detection limit, calculated from the following formula using absorvance value of the dilution solution, to the standard curve.

Absorbance at detection limit

$$= \bar{X} + 3.3 \times \sqrt{\left\{\sum_{i=1}^{6} (X_i - \bar{X})^2\right\}/(6 - 1)}$$

X_i: Individual absorbance values obtained from the dilution solution

$\bar{X}$: The mean of absorbance values obtained from the dilution solution

6: The number of wells in the microplate containing dilution solution

(2) Tetracycline hydrochloride—Serially subculture through 2 passages at 35 to 37°C the test bacteria *Micrococcus luteus* ATCC9341 in a slant culture of test bacteria inoculation media for teceleukin and then dilute this 100-fold by adding sterilized purified water. This is the test bacteria solution. Store the test bacteria solution at 5°C or less and use the solution within 5 days. Dilute the test bacteria solution serially by adding sterilized purified water, add an appropriate amount to 100 mL of normal agar medium for teceleukin, conduct a preliminary test, and determine the amount of tetracycline hydrochloride that shows an inhibition zone corresponding to standard solution containing 0.5 μg (potency) in 1 mL. Add this amount to 100 mL of normal agar medium for teceleukin dissolved and then cooled to 45 to 50°C and mix. Pipet 25 mL of this solution into square Petri dishes (135 × 95 mm) and spread horizontally to solidify. Prepare plates for testing by making an appropriate number of wells in this agar medium. The volume of the test bacteria solution to which 100 mL of normal agar medium for teceleukin has been added is 0.25 to 1.0 mL. Accurately measure an appropriate amount of Tetracycline Hydrochloride RS and dilute accurately with water to make a clear solution with a concentration of 1 mg (potency)/mL. Accurately measure an appropriate amount of this solution and dilute precisely with water to make standard solutions with concentrations of 4, 2, 1 and 0.5 μg (potency)/mL. Separately, dilute Teceleukin (Genetical Recombination) with diluted acetic acid (100) (3 in 1000) if needed, or alternatively concentrate under reduced pressure, to make a sample solution with a protein concentration of 0.8 to 1.2 mg/mL. Accurately measure 25 μL of the sample solution and each standard solution, and add each to the wells in the same test plate. Repeat the same procedure for at least 3 more test plates. Leave the test plates at room temperature for 30 to 60 minutes and then incubate for 16 to 18 hours at 35 to 37°C. Measure the inhibitions zones to a diameter of 0.25 mm. Determine the mean among the test plates for each of the solutions.

Prepare a standard curve by plotting a graph with the concentration of each standard solution in logarithmic scale on the horizontal axis and the diameter of the inhibition zone on the vertical axis. Match the diameter of the inhibition zone of teceleukin from the standard curve and determine A, the concentration of tetracycline hydrochloride. When the amount of tetracycline hydrochloride per mg of protein is determined by the following formula, the amount is not more than 0.7 μg. However, if an inhibition zone is not seen, or is seen but the diameter is smaller than 0.5 μg/mL on the standard curve, A is taken as being 0.5 μg/mL or less.

Amount [μg (potency)] of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8$.HCl) per mg of protein $= A/P$

P: The protein concentration (mg/mL) of the sample solution.

(3) Desmethionyl form—Add water to an appropriate amount of teceleukin to make a sample solution with a protein concentration of about 0.17 mg/mL. Perform the test with 1.2 mL of this solution as directd under Liquid Chromatography <*2.01*> under the following conditions. Determine using automatic integration the peak area, A_2, of teceleukin and the peak area of the desmethionyl form with a relative retention time of about 0.8 relative to teceleukin, A_1. The content of the desmethionyl form is not more than 1.0% when determined using the following formula.

Amount (%) of desmethionyl form $= A_1/(A_1 + A_2) \times 100$

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 280 nm)

Columns: Two stainless steel columns with inside diameters of 7.5 mm and lengths of 7.5 cm connected in sequence and packed with 10 μm synthetic polymer bound to diethylaminoethyl base for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Mix 0.658 g of diethanolamine in 400 mL of water, adjust the pH to 9.0 by adding 1 mol/L hydrochloric acid TS, and then add water to make 500 mL.

Mobile phase B: Add 300 mL of water to 2.6 mL of a pH 6 to 9 amphoteric electrolyte solution and 0.5 mL of a pH 8 to 10.5 amphoteric electrolyte solution, adjust to pH 7 with diluted hydrochloric acid (9 in 100), and then add water to

make 400 mL.

Switching mobile phases and sample injection: Inject the sample solution while running the mobile phase A. Repeatedly inject 10 times a sample solution volume of 0.11 mL followed by a single injection of 100 μL. After injecting the entire volume and running mobile phase A for 60 minutes, switch to mobile phase B. After measuring the sample solution and after running 1 mol/L sodium chloride TS for 10 minutes for postreatment and cleaning of the columns, inject 100 μL of sodium hydroxide TS while running the mobile phase A and then 55 minutes later start injection of the next sample solution.

Flow: Adjust the flow of the mobile phase B so that the retention time for teceleukin is 45 to 65 minutes. Measure the retention time from the point at which the mobile phase is switched to the mobile phase B.

System suitability—

System performance: Dissolve in water a mixture of two kinds of equine heart-derived myoglobin whose isoelectric points are 6.76 and 7.16 to make a concentration of approximately 0.5 mg/mL. Mix together 50 μL of this solution, 50 μL of Teceleukin (Genetical Recombination), and 1.47 mL of water. When 1.2 mL of this solution is run under the above conditions, myoglobin and teceleukin are eluted in this order, and their respective peaks are completely separated.

(4) Dimer—Prepare a sample solution by adding 20 μL of 0.2% sodium laurylsulfate TS to 20 μL of Teceleukin (Genetical Recombination). Perform the test as directed under Liquid Chromatography <*2.01*> using 20 μL of this solution under the following conditions. Determine using automated integration the teceleukin peak area, A_2, and the peak area, A_1, of the dimer with a relative retention time of 0.8 to 0.9 in relation to teceleukin. The amount of the dimer is not more than 1.0% by the following formula.

$$\text{Amount (\%) of dimer} = A_1/(A_1 + A_2) \times 100$$

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 7.5 mm and 60 cm in length, packed with gycol etherified silica gel for liquid chromatography (particle diameter: 10 μm).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 0.1 mol/L sodium phosphate buffer, pH 7.0, to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of teceleukin is between 30 and 40 minutes.

System suitability—

System performance: Add 20 μL of 0.2% sodium lauryl sulfate TS to 20 μL of a solution consisting of 5 mg of carbonic anhydrase and 5 mg of α-lactoalbumin dissolved in 100 mL of water. When 20 μL of this solution is tested under the above conditions, carbonic anhydrase and α-lactoalbumin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: Measure exactly 1.0 mL of the sample solution, add the mobile phase to exactly 20 mL. To exactly 1 mL of this solution add the mobile phase to make exactly 10 mL. When the test is repeated 3 times with 20 μL of this solution under the above conditions, the relative standard deviation of the teceleukin peak area is not more than 7%.

(5) Other related proteins—Perform the test on 5 μL of Teceleukin (Genetical Recombination) as directed under Liquid Chromatography <*2.01*> under the following conditions, and measure the area of each peak using automatic integration. When the amounts are determined by the area percent method, the total amount of peaks other than the teceleukin and solvent peaks is not more than 1.0%.

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 4.6 mm 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 30°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of water and acetonitrile (19:1) (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in acetonitrile (7 in 10,000).

Mobile phase flow: Control the concentration gradient by changing the mobile phase A and mobile phase B as shown in the table below.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 ~ 12	60 → 50	40 → 50
12 ~ 25	50	50
25 ~ 45	50 → 0	50 → 100
45 ~ 50	0	100

Flow rate: 1.0 mL/min.

Time span of measurement: a range that is approximately 1.2-fold the retention time of teceleukin.

System suitability—

System performance: Add 3.8 μL of water and 16.6 μL of polysorbet 80 solution (1 in 100) to 83.6 μL of Teceleukin (Genetical Recombination) and let stand for at least one hour. When 5 μL of this solution is tested by running under the above conditions, there is complete separation between the teceleukin peak and the peak with a relative retention time of about 0.98 in relation to the teceleukin peak.

(6) Acetic acid—Measure exactly 0.25 mL of Teceleukin (Genetical Recombination) and add exactly 0.25 mL of the internal standard solution to make the sample solution. Separately, measure exactly 3 mL of acetic acid (100) and add water to make exactly 100 mL. Take exactly 10 mL of this solution and add water to make exactly 100 mL. Measure exactly 2 mL of this solution and add exactly 2 mL of the internal standard solution to make the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution by Gas Chromatography <*2.02*> under the following conditions. Calculate the ratios of the peak area of acetic acid to that of the internal standard, Q_T and Q_S, and the amount of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination) determined by the following formula is between 2.85 and 3.15 mg.

Amount (mg) of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination)
$= Q_T/Q_S \times 1.5 \times 1.049 \times 2$

1.5: Concentration (μL/mL) of acetic acid (100) in the standard solution
1.049: Density (mg/μL) of acetic acid (100) at 25°C
2: Dilution coefficient

Internal standard solution—Diluted propionic acid (1 in 500).

Operating conditions—

Detector: Hydrogen flame ionization detector.

Column: A glass column with an inside diameter of 1.2 mm and 40 m in length, whose inside is covered with chemically-bound polyethylene glycol for gas chromatography 1.0 μm in thickness.

Column temperature: A constant temperature of about 110°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 8 minutes.

System suitability—

System performance: When 1 μL of the standard solution is run under the above conditions, acetic acid 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 repeatedly run 6 times under the above conditions using 1 μL of standard solution, the relative standard deviation of the ratio of the acetic acid peak area to the internal standard peak area is not more than 5%.

Bacterial endotoxins *<4.01>* Less than 5EU per mg of protein.

Specific activity Accurately measure an appropriate amount of Teceleukin (Genetical Recombination), and add water accurately so that 1 mL contains about 0.1 mg. This is the sample solution. Separately, measure precisely about 25 mg of human serum albumin for assay, dissolve in water, and add water to make 50 mL. Measure exactly an appropriate amount of this solution, and accurately dilute with water to make standard solutions with concentrations of 0.05, 0.10, and 0.15 mg/mL. Accurately measure 1 mL each of the sample solution, the standard solutions, and water, add 2.5 mL of alkaline copper solution, mix, leave for at least 10 minutes to dissolve, add exactly 2.5 mL of water and 0.5 mL of diluted Folin reagent (1 in 2), immediately shake vigorously, and then leave for 30 minutes at 37°C. Perform the test on these solutions, with water as a control, as directd under Ultraviolet-visible Spectrophotometry *<2.24>*, and measure the absorbance at 750 nm. With the concentration of the standard solution as the *x*-axis and the absorbance as the *y*-axis, perform linear regression using their respective reciprocals, and calculate the protein content.

Calculate the ratio of the potency determined by Assay and the protein content.

Assay Accurately measure an appropriate amount of Teceleukin (Genetical Recombination) and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL (estimated value). This is the sample solution. Separately, dissolve Interleukin-2 Reference Substance in 1 mL of sterilized purified water, and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL. This is the standard solution. Add exactly 50 μL of culture medium for assay of teceleukin to all but 8 wells in a microtest plate. Add 50 μL of the sample solution and the standard solution to 2 wells each containing culture medium for assay of teceleukin. From these 4 wells, remove exactly 50 μL and add to 4 other wells containing culture medium for assay of teceleukin. From these 4 wells, remove exactly 50 μL and add to 4 other wells containing culture medium for assay of teceleukin and repeat this procedure to prepare 2 wells that contain each of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 dilutions of the sample and standard solutions. Add 50 μL of the standard solution to each of the 8 empty wells to make maximum uptake controls. Eight wells containing only culture medium for assay of teceleukin serve as the minimum uptake controls. After adding exactly 50 μL of cell suspension solution for teceleukin to each well in a microtest plate, leave for 15 to 17 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. After adding 25 μL of MTT TS to each of the wells in the plate, leave for 4 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. Transfer the culture medium in all of the wells to empty wells in another microtest plate. To each of the empty wells from which the culture medium was removed, add 100 μL of hydrochloric acid-2-propanol TS, and then shake the plates horizontally for 5 minutes to elute the pigment. After returning the transferred culture medium to each original well, perform the test with the solution in each well, determine the difference in absorption at wavelengths of 560 nm and 690 nm, and calculate the mean values of the identical respective solutions in the two wells (dilution solutions of the sample solution and standard solutions) as well as the 8 wells containing the maximum or minimum uptake controls. Prepare standard curves by plotting the values obtained from each dilution solution of the sample solution, with the dilution coefficient of the sample solution on the microtest plates in logarithmic scale on the horizontal axis and the absorbance on the vertical axis. Determine the mean absorbance values of the maximum and minimum uptake controls, find the values on the standard curve, and then calculate the dilution coefficient, D_T. Perform the same plot for the dilution solution of the standard solution, calculate the dilution coefficient, D_S, and then calculate the potency in 1 mL by the following formula.

Teceleukin potency (units) in 1 mL of Teceleukin (Genetical Recombination)
$= S \times D_T/D_S \times d$

S: Concentration of standard solution (units/mL)
d: Dilution coefficient when sample solution prepared

Containers and storage Containers—Tight containers
Storage—Store at −70°C or below.

Teceleukin for Injection (Genetical Recombination)

注射用テセロイキン(遺伝子組換え)

Teceleukin for Injection (Genetical Recombination) is a preparation for injection which is dissolved before use.

It contains not less than 70.0% and not more than 150.0% of the labeled amount of teceleukin (genetical recombination) ($C_{698}H_{1127}N_{179}O_{204}S_8$: 15547.01).

Method of preparation Prepare as directed under Injection, with Teceleukin (Genetical Recombination).

Description Teceleukin for Injection (Genetical Recombination) occurs as a white, light mass or powder.

Identification **(1)** Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample stock solution containing about 200 units per mL (estimate). Proceed as directed in the Identification (1) under Teceleukin (Genetical Recombination).

(2) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. Adjust to pH 8.0 with 1 mol/L hydrochloric acid TS, add water to make 100 mL, and use this solution as the buffer solution for molecular mass determination. Separately, dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of water. To exactly 100 μL of this solution add exactly 100 μL of the buffer solution for molecular mass determination and 10 μL of 2-mercaptoethanol, and heat on a water bath for 5 minutes without allowing any water evaporation from the mixture. After cooling, add exactly 1 μL of bromophenol blue solution (1 in 2000), mix, and use this solution as the sample solution. Proceed as directed in Identification (3) under Teceleukin (Genetical Recombination): a band appears in the range of molecular mass between 14,000 and 16,000.

pH <*2.54*> Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the pH of the solution is between 7.0 and 7.7.

Purity Clarity and color of solution—Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the solution is clear and colorless.

Loss on drying Transfer the content of the vial of Teceleukin for Injection (Genetical Recombination) to a weighing bottle under the atmosphere not exceeding 10% relative humidity, and perform the test as directed in the Water content determination described in the Minimum Requirements for Biological Products: not more than 5%.

Bacterial endotoxins <*4.01*> Less than 5 EU/350,000 units.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test. Calculate as $|M - A| = 0$.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample solution containing a definite concentration of 10 to 50 units/mL (estimate). Proceed as directed in the Assay under Teceleukin (Genetical Recombination), and calculate the amount (unit) of teceleukin in 1 vial by the following formula.

$$\text{Amount (unit) of teceleukin in 1 vial} = S \times D_T/D_S \times d \times 1$$

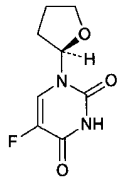
S: Concentration of the standard solution (unit/mL)
d: Dilution coefficlent when sample solution prepared
1: Volume (mL) of the sample solution

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, not exceeding 10°C, avoiding freezing.

Tegafur

テガフール



and enantiomer

$C_8H_9FN_2O_3$: 200.17
5-Fluoro-1-[(2*RS*)-tetrahydrofuran-2-yl]uracil
[*17902-23-7*]

Tegafur, when dried, contains not less than 98.0% of $C_8H_9FN_2O_3$.

Description Tegafur occurs as a white, crystalline powder.

It is soluble in methanol and in acetone, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

Identification **(1)** Prepare the test solution with 0.01 g of Tegafur as directed under Oxygen Flask Combustion Method <*1.06*>, 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 <*1.09*> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Tegafur in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tegafur, previously dried, as directed in the potassium bro-

mide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample with a mixture of methanol and acetone (1:1), filter and dry the crystals, and perform the test with the crystals.

pH <*2.54*> Dissolve 0.5 g of Tegafur in 50 mL of water: the pH of this solution is between 4.2 and 5.2.

Melting point <*2.60*> 166 – 171°C

Purity **(1)** Clarity and color of solution—Dissolve 0.2 g of Tegafur in 10 mL of dilute sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.8 g of Tegafur in 40 mL of water by warming, cool, filter if necessary, 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 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Tegafur in 40 mL of water by warming, cool, filter if necessary, 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 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution in a platinum crucible with 1.0 g of Tegafur according to Method 4, incinerating by ignition between 750°C and 850°C, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Tegafur in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 5 μL each of the sample 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 and ethanol (95) (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

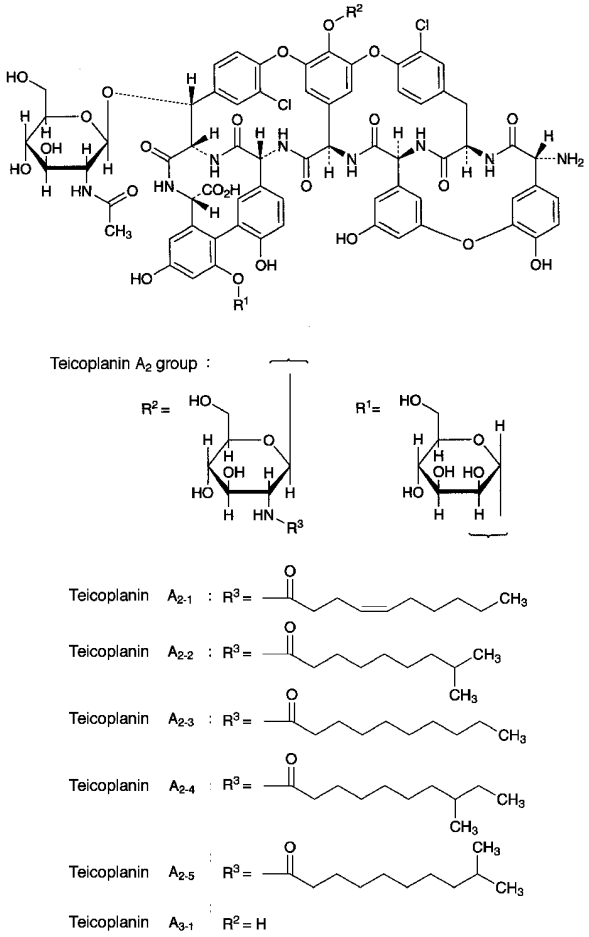
Assay Weigh accurately about 0.15 g of Tegafur, previously dried, place in an iodine bottle, dissolve in 75 mL of water, and add exactly 25 mL of 1/60 mol/L potassium bromate VS. Add rapidly 1.0 g of potassium bromide and 12 mL of hydrochloric acid, stopper the bottle tightly at once, and allow to stand for 30 minutes with occasional shaking. To this solution add 1.6 g of potassium iodide, shake gently, allow to stand for exactly 5 minutes, and titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

Each mL of 1/60 mol/L potassium bromate VS
= 10.01 mg of $C_8H_9FN_2O_3$

Containers and storage Containers—Tight containers.

Teicoplanin

テイコプラニン



Teicoplanin $A_{2\text{-}1}$
$C_{88}H_{95}Cl_2N_9O_{33}$: 1877.64
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-(4*Z*)-dec-4-enoylamino-2-deoxy-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid
[*91032-34-7*]

Teicoplanin $A_{2\text{-}2}$
$C_{88}H_{97}Cl_2N_9O_{33}$: 1879.66
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methylnonanoylamino)-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-

1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetramethano-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-26-7*]

Teicoplanin A_{2-3}
$C_{88}H_{97}Cl_2N_9O_{33}$: 1879.66
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-*β*-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-(2-decanoylamino-2-deoxy-*β*-D-glucopyranosyloxy)-6,11,40,44-tetrahydroxy-42-(*α*-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetramethano-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-36-9*]

Teicoplanin A_{2-4}
$C_{89}H_{99}Cl_2N_9O_{33}$: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-*β*-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methyldecanoylamino)-*β*-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(*α*-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetramethano-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-37-0*]

Teicoplanin A_{2-5}
$C_{89}H_{99}Cl_2N_9O_{33}$: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-*β*-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(9-methyldecanoylamino)-*β*-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(*α*-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetramethano-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-38-1*]

Teicoplanin A_{3-1}
$C_{72}H_{68}Cl_2N_8O_{28}$: 1564.25
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-*β*-D-glucopyranosyloxy)-15-amino-22,31-dichloro-6,11,40,44,56-pentahydroxy-42-(*α*-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetramethano-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [*93616-27-4*]

[*61036-62-2*, Teicoplanin]

Teicoplanin is a mixture of glycopeptide substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.

It contains not less than 900 μg (potency) and not more than 1120 μg (potency) per 1 mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin ($C_{72\text{-}89}H_{68\text{-}99}Cl_2N_{8\text{-}9}O_{28\text{-}33}$).

Description Teicoplanin occurs as a white to light yellowish white powder.

It is freely soluble in water, sparingly soluble in *N*,*N*-dimethylformamide, and practically insoluble in acetonitrile, in methanol, in ethanol (95), in acetone, in acetic acid (100) and in diethyl ether.

Identification **(1)** To 1 mL of a solution of Teicoplanin (1 in 100) add 2 mL of ninhydrin TS, and warm for 5 minutes: a blue-purple color develops.

(2) To 1 mL of a solution of Teicoplanin (3 in 100) add slowly 2 mL of anthrone TS, and shake gently: a dark brown color develops.

(3) Determine the infrared absorption spectra of Teicoplanin and Teicoplanin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the spectrum of Teicoplanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH *<2.54>* Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

Content ratio of the active principle Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the sum of peak areas of teicoplanin A_2 group, S_a, the sum of peak areas of teicoplanin A_3 group, S_b, and the sum of peak areas of other contents, S_c from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A_2 group, teicoplanin A_3 group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

The elution order of each content and the relative retention time of each content to the retention time of teicoplanin $A_{2\text{-}2}$ are shown in the following table.

Name of content	Elution order	Relative retention time
teicoplanin A_3 group		≦0.42
teicoplanin $A_{3\text{-}1}$	1	0.29
teicoplanin A_2 group		0.42<, ≦1.25
teicoplanin $A_{2\text{-}1}$	2	0.91
teicoplanin $A_{2\text{-}2}$	3	1.00
teicoplanin $A_{2\text{-}3}$	4	1.04
teicoplanin $A_{2\text{-}4}$	5	1.17
teicoplanin $A_{2\text{-}5}$	6	1.20
others		1.25<

Content ratio (%) of teicoplanin A_2 group
$= S_a/(S_a + 0.83S_b + S_c) \times 100$

Content ratio (%) of teicoplanin A_3 group
$= 0.83S_b/(S_a + 0.83S_b + S_c) \times 100$

Content ratio (%) of others
$= S_c/(S_a + 0.83S_b + S_c) \times 100$

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Mobile phase B: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 550 mL of water, add 1400 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Flowing of the mobile phase: Flow mobile phase A for 10 minutes before injection. After injection, control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 32	100 → 70	0 → 30
32 – 40	70 → 50	30 → 50
40 – 42	50 → 100	50 → 0

Flow rate: 1.8 mL per minute.

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin $A_{2\text{-}2}$, beginning after the solvent peak.

System suitability—

Test for required detection: Confirm that peak height of teicoplanin $A_{2\text{-}2}$ obtained from the sample solution is equivalent to 90% of the full scale.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the symmetry factor of the peak of teicoplanin $A_{3\text{-}1}$ is not more than 2.2.

System repeatability: When the test is repeated 3 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of teicoplanin $A_{2\text{-}2}$ is not more than 2.0%.

Purity **(1)** Clarity and color of solution—Being specified separately.

(2) Sodium chloride—Weigh accurately about 0.5 g of Teicoplanin, dissolve in 50 mL of water, titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS), and calculate an amount of sodium chloride: not more than 5.0%.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

(3) Heavy metals ⟨*1.07*⟩—Being specified separately.

(4) Arsenic ⟨*1.11*⟩—Being specified separately.

(5) Residual solvents ⟨*2.46*⟩—Weigh accurately about 0.1 g of Teicoplanin, dissolve in *N*,*N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g each of methanol and acetone, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 4 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine the peak area of methanol, A_1, and the peak area of acetone, A_2, obtained from the sample solution, and the peak area of methanol, A_{S1}, and the peak area of acetone, A_{S2}, obtained from the standard solution by the automatic integration method, and calculate the amounts of methanol and acetone by the following formula: not more than 0.5% and not more than 1.0%, respectively.

Amount (%) of methanol
$= M_{S1} \times A_1/A_{S1} \times 0.001 \times 1/M_T \times 100$

Amount (%) of acetone
$= M_{S2} \times A_2/A_{S2} \times 0.001 \times 1/M_T \times 100$

M_{S1}: Amount (g) of methanol
M_{S2}: Amount (g) of acetone
M_T: Amount (g) of Teicoplanin

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A glass column 2 mm in inside diameter and 3 m in length, packed with graphite carbon for gas chromatography, 150 to 180 μm in particle diameter, coated with 0.1% of polyethylene glycol esterified.

Column temperature: Inject the sample at a constant temperature of about 70°C, maintain the temperature for 4 minutes, then program to increase the temperature at the rate of 8°C per minute to 210°C.

Detector temperature: A constant temperature of about 240°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention times of methanol and acetone are about 2 minutes and 5 minutes, respectively.

System suitability—

Test for required detection: Confirm that the peak height of acetone obtained from 4 μL of the standard solution is equivalent to about the full scale.

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, methanol and acetone are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone is not more than 3%.

Water ⟨*2.48*⟩ Not more than 15.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.75 EU/mg (potency).

Blood pressure depressant Being specified separately.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Teicoplanin RS equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of this solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 μg (potency) and 40 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

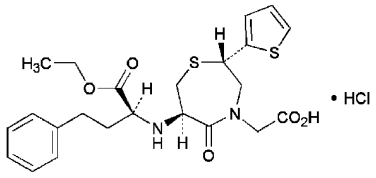
(iv) Sample solutions—Weigh accurately an amount of Teicoplanin equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 μg (potency) and 40 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 5°C.

Temocapril Hydrochloride

テモカプリル塩酸塩



$C_{23}H_{28}N_2O_5S_2$.HCl: 513.07

2-[(2*S*,6*R*)-6-{[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino}-5-oxo-2-(thiophen-2-yl)-2,3,6,7-tetrahydro-1,4-thiazepin-4(5*H*)-yl] acetic acid monohydrochloride

[*110221-44-8*]

Temocapril Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{23}H_{28}N_2O_5S_2$.HCl, calculated on the anhydrous basis.

Description Temocapril Hydrochloride occurs as a white crystalline powder.

It is freely soluble in ethanol (99.5), and vey slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Temocapril Hydrochloride as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +60 – +64° (0.2 g, calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Temocapril 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).

(2) Related substances—Dissolve 50 mg of Temocapril Hydrochloride in 100 mL of diluted acetonitrile (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetonitrile (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than temocapril obtained from the sample solution is not larger than 1/5 times the peak area of temocapril from the standard solution, and the total area of the peaks other than temocapril from the sample solution is not larger than 1/2 times the peak area of temocapril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6.0 mm in inside 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 diluted phosphoric acid (1 in 500) and acetonitrile (63:37).

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

Time span of measurement: About 4 times as long as the retention time of temocapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add diluted acetonitrile (1 in 2) to make exactly 10 mL. Confirm that the peak area of temocapril obtained with 10 μL of this solution is equivalent to 7 to 13% 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 temocapril are not less than 7000 and not more than 1.5, respectively.

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

(3) Residual solvent—Being specified separately.

Water <2.48> Not more than 1.0% (0.3 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Temocapril Hydrochloride, dissolve in 80 mL of a mixture of acetic anhydrate and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.31 mg of $C_{23}H_{28}N_2O_5S_2$.HCl

Containers and storage Containers—Well-closed containers.

Temocapril Hydrochloride Tablets

テモカプリル塩酸塩錠

Temocapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl: 513.07).

Method of preparation Prepare as directed under Tablets, with Temocapril Hydrochloride.

Identification To an amount of powdered Temocapril Hydrochloride Tablets, equivalent to 2.5 mg of Temocapril Hydrochloride according to the labeled amount, add 25 mL of diluted acetonitrile (1 in 2), shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 232 nm and 236 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Temocapril Hydrochloride Tablets add exactly 20 mL of diluted acetonitrile (1 in 2), and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. Pipet V mL of the supernatant liquid equivalent to about 0.8 mg of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl), add exactly 2 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of temocapril hydrochloride for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Temocapril Hydrochloride), dissolve in diluted acetonitrile (1 in 2) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 2 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of temocapril to that of the internal standard.

Amount (mg) of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl)
$= M_S \times Q_T/Q_S \times 1/V \times 2/5$

M_S: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 3000).

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 under the above operating conditions, temocapril and the internal standard 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Temocapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Temocapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 1.1 μg of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of temocapril hydrochloride for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, and add 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. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak area, A_T and A_S, of temocapril of both solutions.

Dissolution rate (%) with respect to the labeled amount of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (43:32).

Flow rate: Adjust the flow rate so that the retention time of temocapril is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of temocapril are not less than 9000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temocapril is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Temocapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl), add exactly 20 mL of the internal standard solution, and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of temocapril hydrochloride for assay (separately determine the water <2.48> in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of temocapril to that of the internal standard.

Amount (mg) of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 3000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6.0 mm in inside 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 diluted phosphoric acid (1 in 500) and acetonitrile (63:37).

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

System suitability—

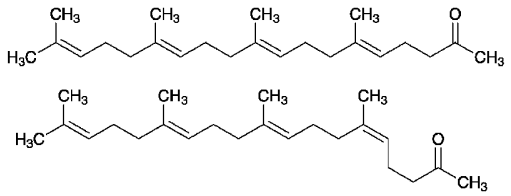
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Teprenone

テプレノン



$C_{23}H_{38}O$: 330.55
(5*E*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one
(5*Z*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one
[*6809-52-5*]

Teprenone contains not less than 97.0% and not more than 101.0% of $C_{23}H_{38}O$.

Teprenone is comprised of mono-cis and all-trans isomers, with their ratio being about 2:3.

Description Teprenone occurs as a colorless to slightly yellowish clear oily liquid, with slight, characteristic odor.

It is miscible with ethanol (99.5), with ethyl acetate and with hexane.

It is practically insoluble in water.

It is oxidized by air, and gradually turns yellow.

Identification (1) To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 1 mL of a solution of phosphomolybdic acid *n*-hydrate in acetic acid (100) (1 in 100), heat in a water bath for 5 minutes, and continue heating with addition of 5 to 6 drops of sulfuric acid: blue to bluish green color develops.

(2) To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 2 mL of 2,4-dinitrophenylhydrazine TS, and shake: a yellow to orange-yellow precipitate is formed.

(3) Determine the infrared absorption spectrum of Teprenone as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Teprenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20}: 1.485 – 1.491

Specific gravity <2.56> d_{20}^{20}: 0.882 – 0.890

Purity (1) Clarity and color of solution—To 1.0 mL of Teprenone add 9 mL of ethanol (99.5) and shake: the solution is clear, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.02.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Teprenone 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 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area from the sample

solution by the automatic integration method and calculate the amounts of them by the area percentage method: the peak area of the di-cis isomer of teprenone, having the relative retention time of about 0.8 with respect to the all-trans isomer of teprenone, is not more than 0.5%, and each area of the peaks for the mono-cis and all-trans isomers of the teprenone and for those other than mentioned above is not more than 0.2%. Furthermore, the total area of the peaks other than the mono-cis, all-trans and di-cis isomers of teprenone is not more than 1.0%.

Operating conditions—

Detector, column, column temperature, carrier gas and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time for the all-trans isomer of teprenone beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add hexane to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add hexane to make exactly 10 mL. Confirm that the sum of the peak areas of the mono-cis and all-trans isomers of teprenone obtained from 3 μL of this solution is 7 to 13% of the peak areas of the mono-cis and all-trans isomers of teprenone from 3 μL of the solution for system suitability test.

System performance: When the procedure is run with 3 μL of the solution for system suitability test under the above operating conditions, the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between these peaks being not less than 1.1.

System repeatability: When the test is repeated 6 times with 3 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone is not more than 3.0%.

(4) Residual solvent—Being specified separately.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Isomer ratio Dissolve 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having retention times of about 18 minutes, where A_a is the peak of the mono-cis isomer, having the shorter retention time, and A_b is the peak area of the all-trans isomer, having the longer retention time: A_a/A_b is 0.60 to 0.70.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Purity (3).

Assay Weigh accurately about 50 mg each of Teprenone and Teprenone RS, dissolve each in exactly 5 mL of the internal standard solution, add ethyl acetate to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of teprenone (sum of the peak areas of mono-cis and all-trans isomers) to that of the internal standard.

$$\text{Amount (mg) of teprenone } (C_{23}H_{38}O) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Teprenone RS

Internal standard solution—A solution of di-*n*-butyl phthalate in ethyl acetate (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 2 m in length, packed with 149 to 177 μm siliceous earth for gas chromatography coated in 5% with polyethylene glycol 2-nitroterephthalate.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of the peak of the all-trans isomer of teprenone, the larger of the two main peaks, having the retention time about 18 minutes, becomes 19 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between the mono-cis and all-trans isomers being not less than 1.1.

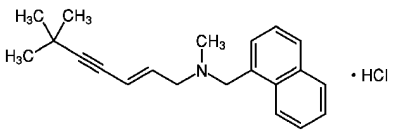
System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight Containers.

Storage—Under Nitrogen atmosphere at 2 to 8°C.

Terbinafine Hydrochloride

テルビナフィン塩酸塩



$C_{21}H_{25}N.HCl$: 327.89

(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine monohydrochloride

[*78628-80-5*]

Terbinafine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{25}N.HCl$.

Description Terbinafine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.

The pH of a solution of 1.0 g of Terbinafine Hydrochlo-

ride in 1000 mL of water is 3.5 to 4.5.

Melting point: about 205°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Terbinafine Hydrochloride in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Terbinafine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Terbinafine Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Terbinafine 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).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Terbinafine Hydrochloride in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of a dimer, having the relative retention time of about 1.7 with respect to terbinafine obtained from the sample solution is not larger than 1/2 times the peak area of terbinafine from the standard solution, the area of the peak other than terbinafine and the dimer from the sample solution is not larger than the peak area of terbinafine from the standard solution, and the total area of the peaks other than terbinafine is not larger than 3 times the peak area of terbinafine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3 mm in inside 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: To 700 mL of a mixture of methanol and acetonitrile (3:2) add 300 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.

Mobile phase B: To 950 mL of a mixture of methanol and acetonitrile (3:2) add 50 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 4	100	0
4 – 25	100 → 0	0 → 100
25 – 30	0	100

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

Time span of measurement: About 2 times as long as the retention time of terbinafine, beginning after the solvent peak.

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 20 mL. Confirm that the peak area of terbinafine obtained with 20 μL of this solution is equivalent to 18 to 32% of that with 20 μL of the standard solution.

System performance: Dissolve 20 mg of Terbinafine Hydrochloride in 20 mL of a mixture of water and acetonitrile (1:1), and irradiate under a short-wave lamp (main wavelength: 254 nm) for 1 hour. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peak of cis-terbinafine, having the relative retention time of about 0.94 with respect to terbinafine, and the peak of terbinafine is not less than 2.0.

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

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.26 g of Terbinafine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.79 mg of $C_{21}H_{25}N.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Terbinafine Hydrochloride Cream

テルビナフィン塩酸塩クリーム

Terbinafine Hydrochloride Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$: 327.89).

Method of preparation Prepare as directed under Creams, with Terbinafine Hydrochloride.

Identification To quantity of Terbinafine Hydrochloride Cream, equivalent to 10 mg of Terbinafine Hydrochloride,

according to the labeled amount, dissolve in 20 mL of 2-propanol, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 20 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and 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 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same *R*f value with the spot from the standard solution.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Cream, equivalent to about 10 mg of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$), dissolve in 2-propanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in 2-propanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the terbinafine peak areas, A_T and A_S, of both solutions.

Amount (mg) of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$) $= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 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: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability—

System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbinafine Hydrochloride Solution

テルビナフィン塩酸塩液

Terbinafine Hydrochloride Solution is a liquid for external use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$: 327.89).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Terbinafine Hydrochloride.

Identification To a volume of Terbinafine Hydrochloride Solution, equivalent to 10 mg of Terbinafine Hydrochloride according to the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay 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 <*2.03*>. Spot 5 μL each of the sample solution and 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 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same *R*f value with the spot from the standard solution.

pH Being specified separately.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Solution, equivalent to about 10 mg of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$), add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the terbinafine peak areas, A_T and A_S, of both solutions.

Amount (mg) of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$) $= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 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: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability—

System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbinafine Hydrochloride Spray

テルビナフィン塩酸塩スプレー

Terbinafine Hydrochloride Spray contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$: 327.89).

Method of preparation Prepare as directed under Pump Sprays for Cutaneous Application, with Terbinafine Hydrochloride.

Identification To an amount of Terbinafine Hydrochloride Spray, equivalent to 10 mg of Terbinafine Hydrochloride, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay 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 ⟨*2.03*⟩. Spot 5 μL each of the sample solution and 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 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same *R*f value with the spot from the standard solution.

pH Being specified separately.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Spray, equivalent to about 10 mg of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$), dissolve in methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the terbinafine peak areas, A_T and A_S, of both solutions.

Amount (mg) of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$) $= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 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: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability—

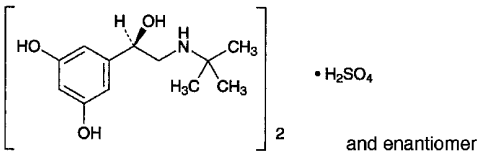
System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, terphenyl and terbinafine are eluted in this order with the resolution between these peaks being not less than 6.

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

Containers and storage Containers—Tight containers.

Terbutaline Sulfate

テルブタリン硫酸塩



$(C_{12}H_{19}NO_3)_2.H_2SO_4$: 548.65

5-[(1*RS*)-2-(1,1-Dimethylethylamino)-1-hydroxyethyl]benzene-1,3-diol hemisulfate

[*23031-32-5*]

Terbutaline Sulfate contains not less than 98.5% of $(C_{12}H_{19}NO_3)_2.H_2SO_4$, calculated on the anhydrous basis.

Description Terbutaline Sulfate is white to slightly brownish white crystals or crystalline powder. It is odorless or has a faint odor of acetic acid.

It is freely soluble in water, and practically insoluble in acetonitrile, in ethanol (95), in acetic acid (100), in chloroform, and in diethyl ether.

It is gradually colored by light and by air.

Melting point: about 255°C (with decomposition).

Identification **(1)** Dissolve 1 mg of Terbutaline Sulfate in 1 mL of water, and add 5 mL of Tris buffer solution, pH 9.5, 0.5 mL of 4-aminoantipyrine solution (1 in 50) and 2 drops of potassium hexacyanoferrate (III) solution (2 in 25): a reddish purple color is produced.

(2) Determine the absorption spectrum of a solution of

Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths. This maximum can be biphasic.

(3) A solution of Terbutaline Sulfate (1 in 50) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve Terbutaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 4.8.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Terbutaline Sulfate in 10 mL of water: the solution is clear and colorless or slightly yellow.

(2) Chloride <*1.03*>—Perform the test with 2.0 g of Terbutaline Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.004%).

(3) Acetic acid—Dissolve 0.50 g of Terbutaline Sulfate in a solution of phosphoric acid (59 in 1000) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 1.50 g of acetic acid (100) in a solution of phosphoric acid (59 in 1000) to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with a solution of phosphoric acid (59 in 1000) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following operating conditions. Measure the peak areas, A_T and A_S, of acetic acid for the two solutions: A_T is not larger than A_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with 10% of macrogol 6000 on 180- to 250-μm terephthalic acid for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Carrier gas: Nitrogen.

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

System suitability—

System performance: Mix 0.05 g each of acetic acid (100) and propionic acid in 100 mL of diluted phosphoric acid (59 in 1000). When the procedure is run with 2 μL of this solution under the above conditions, acetic acid and propionic acid 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 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 3.0%.

(4) 3,5-Dihydroxy-ω-tert-butylaminoacetophenone sulfate—Dissolve 0.50 g of Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and perform the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at a wavelength of 330 nm does not exceed 0.47.

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Terbutaline Sulfate as directed under 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 <*1.11*>—Prepare the test solution with 1.0 g of Terbutaline Sulfate according to method 3, and perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

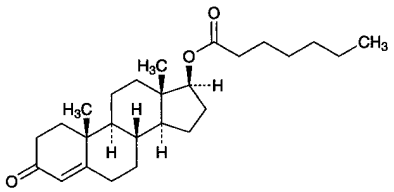
Assay Weigh accurately about 0.5 g of Terbutaline Sulfate, dissolve in 50 mL of a mixture of acetonitrile and acetic acid (100) (1:1) by stirring and warming. Allow to cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration, substituting a saturated solution of potassium chloride in methanol for the internal fluid).

Each mL of 0.1 mol/L perchloric acid VS
= 54.87 mg of $(C_{12}H_{19}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Testosterone Enanthate

テストステロンエナント酸エステル



$C_{26}H_{40}O_3$: 400.59
3-Oxoandrost-4-en-17β-yl heptanoate
[*315-37-7*]

Testosterone Enanthate, when dried, contains not less than 95.0% and not more than 105.0% of $C_{26}H_{40}O_3$.

Description Testosterone Enanthate occurs as white to pale yellow crystals, crystalline powder or a pale yellow-brown, viscous liquid. It is odorless or has a slight, characteristic odor.

It is very soluble in ethanol (95), in 1,4-dioxane and in diethyl ether, and practically insoluble in water.

Melting point: about 36°C.

Identification Heat 25 mg of Testosterone Enanthate with 2 mL of a solution of potassium hydroxide in methanol (1 in 100) under a reflux condenser on a water bath for 1 hour, cool, and add 10 mL of water. Collect the produced precipitate by suction, wash with water until the last washing is neutral, and dry the precipitate in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the precipitate melts <*2.60*> between 151°C and 157°C.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +77 – +88° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Acidity—Dissolve 0.5 g of Testosterone Enanthate in 10 mL of ethanol (95) which has previously been rendered neutral to bromothymol blue TS, and add 2 drops of bromothymol blue TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is light blue.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, in vacu-

um, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.1 g of Testosterone Enanthate, previously dried, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ with this solution. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm.

$$\text{Amount (mg) of } C_{26}H_{40}O_3 = A/426 \times 100{,}000$$

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 30°C.

Testosterone Enanthate Injection

テストステロンエナント酸エステル注射液

Testosterone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of testosterone enanthate ($C_{26}H_{40}O_3$: 400.59).

Method of preparation Prepare as directed under Injections, with Testosterone Enanthate.

Description Testosterone Enanthate Injection is a clear, colorless or pale yellow oily liquid.

Identification Measure a volume of Testosterone Enanthate Injection, equivalent to 0.05 g of Testosterone Enanthate according to the labeled amount, add 8 mL of petroleum ether, and extract with three 10-mL portions of diluted acetic acid (100) (7 in 10). Combine the extracts, wash with 10 mL of petroleum ether, add 0.5 mL of diluted sulfuric acid (7 in 10) to 0.1 mL of the extract, and heat on a water bath for 5 minutes. Cool, and add 0.5 mL of iron (III) chloride-acetic acid TS: the color of the solution is blue.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to Method 2: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure accurately a volume of Testosterone Enanthate Injection, equivalent to about 25 mg of testosterone enanthate ($C_{26}H_{40}O_3$), and dissolve in chloroform to make exactly 25 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Testosterone Propionate RS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 45 minutes. Determine the absorbances, A_T and A_S, of these solutions at 380 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution obtained by proceeding with 5 mL of chloroform as the blank.

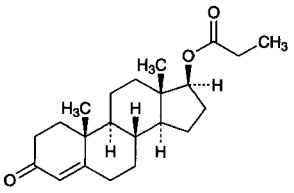
$$\text{Amount (mg) of testosterone enanthate } (C_{26}H_{40}O_3) = M_S \times A_T/A_S \times 1.163$$

M_S: Amount (mg) of Testosterone Propionate RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Testosterone Propionate

テストステロンプロピオン酸エステル



$C_{22}H_{32}O_3$: 344.49
3-Oxoandrost-4-en-17β-yl propanoate
[*57-85-2*]

Testosterone Propionate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

Description Testosterone Propionate occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Testosterone Propionate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Testosterone Propionate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Testosterone Propionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Testosterone Propionate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +83 – +90° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 118 – 123°C

Purity Related substances—Dissolve 40 mg of Testosterone Propionate in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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 and diethylamine (19:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately each about 10 mg of Testosterone Propionate and Testosterone Propionate RS, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of these solutions add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of testosterone propionate to that of the internal standard.

$$\text{Amount (mg) of } C_{22}H_{32}O_3 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Testosterone Propionate RS

Internal standard solution—A solution of progesterone in methanol (9 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of testosterone propionate 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 testosterone propionate 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of testosterone propionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Testosterone Propionate Injection

テストステロンプロピオン酸エステル注射液

Testosterone Propionate Injection is an oily solution for injection.

It contains not less than 92.5% and not more than 107.5% of the labeled amount of testosterone propionate ($C_{22}H_{32}O_3$: 344.49).

Method of preparation Prepare as directed under Injections, with Testosterone Propionate.

Description Testosterone Propionate Injection is a clear, colorless or pale yellow oily liquid.

Identification Dissolve the residue obtained as directed in the procedure in the Assay in exactly 20 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Testosterone Propionate 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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 and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f values of the principal spot with the sample solution and of the spot with the standard solution are not different each other.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to Method 2: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Chromatographic tube A glass tube about 1 cm in inside diameter and about 18 cm in length, with a glass filter (G3) at the lower end.

(ii) Chromatographic column To about 2 g of silica gel for liquid chromatography add 5 mL of dichloromethane, and mix gently. Transfer and wash into the chromatographic tube with the aid of dichloromethane, allow to elute the dichloromethane through the column, and put a filter paper on the upper end of the silica gel.

(iii) Standard solution Weigh accurately about 10 mg of Testosterone Propionate RS, previously dried at 105°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 and methanol to make 20 mL.

(iv) Sample stock solution To exactly a volume of Testosterone Propionate Injection, equivalent to about 20 mg of testosterone propionate ($C_{22}H_{32}O_3$), add dichloromethane to make 20 mL.

(v) Procedure Transfer exactly 2 mL of the sample stock solution into the chromatographic column, and elute to the upper surface of the silica gel. Wash the inner surface of the chromatographic tube with 15 mL of dichlorometh-

ane, elute to the upper surface of the silica gel, and discard the effluent. Elute 15 mL of a mixture of dichloromethane and methanol (39:1), discard the first 5 mL of the effluent, and collect the subsequent effluent. Wash the lower part of the column with a few amount of dichloromethane, combine the washings and the effluent, and evaporate the solvent under reduced pressure. Dissolve the residue so obtained with methanol to make 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Assay under Testosterone Propionate.

$$\text{Amount (mg) of testosterone propionate } (C_{22}H_{32}O_3) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Testosterone Propionate RS

Internal standard solution—A solution of progesterone in methanol (9 in 100,000).

Containers and storage Containers—Hermetic containers.

Freeze-dried Tetanus Antitoxin, Equine

乾燥破傷風ウマ抗毒素

Freeze-dried Tetanus Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains tetanus antitoxin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Tetanus Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Tetanus Antitoxin, Equine, becomes a clear, colorless to light yellow-brown liquid or slightly white-turbid liquid on addition of solvent.

Adsorbed Tetanus Toxoid

沈降破傷風トキソイド

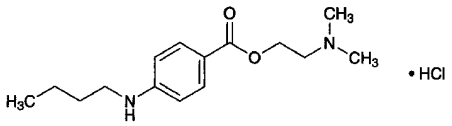
Adsorbed Tetanus Toxoid is a liquid for injection containing tetanus toxoid prepared by treating tetanus toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Tetanus Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Tetanus Toxoid becomes a uniform white-turbid liquid on shaking.

Tetracaine Hydrochloride

テトラカイン塩酸塩



$C_{15}H_{24}N_2O_2.HCl$: 300.82
2-(Dimethylamino)ethyl 4-(butylamino)benzoate monohydrochloride
[*136-47-0*]

Tetracaine Hydrochloried, when dried, contains not less than 98.5% of $C_{15}H_{25}N_2O_2.HCl$.

Description Tetracaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste followed by a sense of numbness on the tongue.

It is very soluble in formic acid, freely soluble in water, soluble in ethanol (95), sparingly soluble in ethanol (99.5), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

A solution of Tetracaine Hydrochloride (1 in 10) is neutral.

Melting point: about 148°C.

Identification **(1)** Dissolve 0.5 g of Tetracaine Hydrochloride in 50 mL of water, add 5 mL of ammonia TS, shake, and allow to stand in a cold place. Collect the precipitate, wash with water until the washings is neutral, and dry in a desiccator (silica gel) for 24 hours: it melts <*2.60*> between 42°C and 44°C.

(2) Dissolve 0.1 g of Tetracaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: a crystalline precipitate is produced. Collect the precipitate, recrystallize from water, and dry at 80°C for 2 hours: it melts <*2.60*> between 130°C and 132°C.

(3) Determine the absorption spectrum of a solution of Tetracaine Hydrochloride in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tetracaine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

Purity Heavy metals <*1.07*>—Proceed with 1.0 g of Tetracaine 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).

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

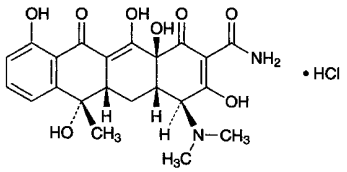
Assay Weigh accurately about 0.5 g of Tetracaine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 80 mL of acetic anhydride, allow to stand at 30°C on a water bath for 15 minutes, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.08 mg of $C_{15}H_{24}N_2O_2$.HCl

Containers and storage Containers—Tight containers.

Tetracycline Hydrochloride

テトラサイクリン塩酸塩



$C_{22}H_{24}N_2O_8$.HCl: 480.90
(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-Dimethylamino-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride
[*64-75-5*]

Tetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces aureofaciens*.

It contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Tetracycline Hydrochloride is expressed as mass (potency) of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8$.HCl).

Description Tetracycline Hydrochloride occurs as a yellow to pale brownish yellow crystalline powder.

It is freely soluble in water, and sparingly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Tetracycline Hydrochloride (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tetracycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 1.0 g of Tetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 1.8 and 2.8.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Tetracycline 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 <*1.11*>—Prepare the test solution with 1.0 g of Tetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Tetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas of each peak by the automatic integration method: each peak area other than tetracycline from the sample solution is not larger than the peak area of tetracycline from the standard solution, and the total area of the peaks other than tetracycline from the sample solution is not larger than 3 times of the peak area of tetracycline 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.

Time span of measurement: About 7 times as long as the retention time of tetracycline beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 3 mL of the standard solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and confirm that the peak area of tetracycline obtained from 20 μL of this solution is equivalent to 1 to 5% of that from 20 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.3% (1.0 g).

Assay Weigh accurately an amount of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS, equivalent to about 25 mg (potency), and dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area, A_T and A_S, of tetracycline of each solution.

Amount [μg (potency)] of $C_{22}H_{24}N_2O_8$.HCl
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Tetracycline Hydrochloride RS

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with stylene-divinylbenzene copolymer for liquid chromatography (0.01 μm in pore diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogenphosphate, 2.0 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, adjust to pH 9.0 with sodium hydroxide TS, add 90.0 g of *t*-butanol, and add water to make 1000 mL.

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

System suitability—

System performance: Dissolve 0.05 g of Tetracycline Hydrochloride RS in water to make 25 mL. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the retention time of 4-epitetracycline is about 3 minutes, and 4-epitetracycline and tetracycline 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thallium (^{201}Tl) Chloride Injection

塩化タリウム (^{201}Tl) 注射液

Thallium (^{201}Tl) Chloride Injection is an aqueous solution for injection

It contains thallium-201 (^{201}Tl) in the form of thallous chloride.

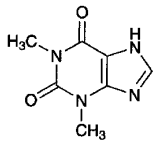
It conforms to the requirements of Thallium (^{201}Tl) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Thallium (^{201}Tl) Chloride Injection is a clear, colorless liquid.

Theophylline

テオフィリン



$C_7H_8N_4O_2$: 180.16

1,3-Dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione

[*58-55-9*]

Theophylline, when dried, contains not less than 99.0% of $C_7H_8N_4O_2$.

Description Theophylline occurs as white crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Theophylline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 271 – 275°C

Purity **(1)** Acidity—To 0.5 g of Theophylline add 75 mL of water, 2.0 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red TS: a yellow color develops.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Theophylline 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 <*1.11*>—Prepare the test solution with 1.0 g of Theophylline according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Theophylline in 3 mL of *N*,*N*-dimethylformamide, add 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 10 μL each of the sample 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, chloroform, methanol, 1-butanol and ammonia solution (28) (3:3:2:2:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

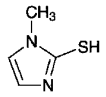
Assay Weigh accurately about 0.25 g of Theophylline, previously dried, and dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.02 mg of $C_7H_8N_4O_2$

Containers and storage Containers—Well-closed containers.

Thiamazole

チアマゾール



$C_4H_6N_2S$: 114.17
1-Methyl-1*H*-imidazole-2-thiol
[*60-56-0*]

Thiamazole, when dried, contains not less than 98.0% of $C_4H_6N_2S$.

Description Thiamazole occurs as white to pale yellowish white crystals or crystalline powder. It has a faint, characteristic odor, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

The pH of the solution (1 in 50) is between 5.0 and 7.0.

Identification **(1)** Dissolve 5 mg of Thiamazole in 1 mL of water, shake with 1 mL of sodium hydroxide TS, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it changes to blue.

(2) To 2 mL of a solution of Thiamazole (1 in 200) add 1 mL of sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 5): a deep blue color develops.

Melting point <*2.60*> 144 – 147°C

Purity **(1)** Selenium—Proceed with 0.10 g of Thiamazole as directed under Oxygen Flask Combustion Method <*1.06*>, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 25 mL of water, and combine the washings with the test solution. Boil gently for 10 minutes, cool to room temperature, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), heat to dissolve on a water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. To 2 mL of this solution, exactly measured, add diluted nitric acid (1 in 60) to make exactly 50 mL, and use this solution as the standard solution. Pipet 40 mL each of the sample solution and standard solution into separate beakers, and adjust each solution with ammonia solution (28) to a pH of 1.8 to 2.2. To each solution add 0.2 g of hydroxylammonium chloride, shake gently to dissolve. To these solutions add 5 mL of a solution prepared by dissolving 0.10 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylammonium chloride in 0.1 mol/L hydrochloric acid TS to make 100 mL, shake, and allow to stand for 100 minutes. Transfer these solutions to corresponding separators, rinse the beakers with 10 mL of water, combine the rinsings in the respective separators, shake well with 5.0 mL of cyclohexane for 2 minutes, and extract. Centrifuge the cyclohexane extracts to remove any water remaining in these solutions. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 40 mL of diluted nitric acid (1 in 60) in the same manner as the blank. The absorbance of of the sample solution at the wavelength of maximum absorbance at about 378 nm does not exceed the absorbance of the standard solution.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Thiamazole 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 <*1.11*>—Prepare the test solution with 1.0 g of Thiamazole according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Thiamazole, previously dried, dissolve in 75 mL of water, add 15 mL of 0.1 mol/L sodium hydroxide VS from a burette, and add 30 mL of 0.1 mol/L silver nitrate VS with stirring. Add 1 mL of bromothymol blue TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS, until a persistent blue-green color is produced. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.42 mg of $C_4H_6N_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Thiamazole Tablets

チアマゾール錠

Thiamazole Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of thiamazole ($C_4H_6N_2S$: 114.17).

Method of preparation Prepare as directed under Tablets, with Thiamazole.

Identification **(1)** To a quantity of powdered Thiamazole Tablets, equivalent to 0.05 g of Thiamazole according to the labeled amount, add 20 mL of hot ethanol (95), shake for 15 minutes, filter, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water, filter if necessary, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of sodium hydroxide TS, shake, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it changes to blue.

(2) With 2 mL of the sample solution obtained in (1), proceed as directed in the Identification (2) under Thiamazole.

Assay Weigh accurately and powder not less than 20 Thiamazole Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of thiamazole ($C_4H_6N_2S$), add 80 mL of water, shake for 15 minutes, add

water to make exactly 100 mL, and centrifuge. Filter, discard the first 20 mL of the filtrate, pipet 50 mL of the subsequent filtrate, add 1 mL of bromothymol blue TS, and if a blue color develops, neutralize with 0.1 mol/L hydrochloric acid VS until the color of the solution changes to green. To this solution add 4.5 mL of 0.1 mol/L sodium hydroxide VS from a burette, add 15 mL of 0.1 mol/L silver nitrate VS while stirring, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS. Continue the titration until a persistent blue-green color is produced, and determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.42 mg of $C_4H_6N_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Thiamine Chloride Hydrochloride

Vitamin B_1 Hydrochloride

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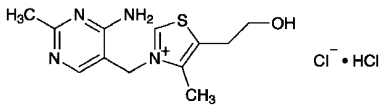


$C_{12}H_{17}ClN_4OS.HCl$: 337.27
3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride
[*67-03-8*]

Thiamine Chloride Hydrochloride contains not less than 98.5% of $C_{12}H_{17}ClN_4OS.HCl$, calculated on the anhydrous basis.

Description Thiamine Chloride Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 245°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Thiamine Chloride Hydrochloride (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(2) Determine the absorption spectrum of a solution of Thiamine Chloride Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Thiamine Chloride Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Thiamine Chloride Hydrochloride, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum, or the spectrum of Thiamine Chloride Hydrochloride RS previously driedat 105°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in water, evaporating to dryness, and drying at 105°C for 2 hours.

(4) A solution of Thiamine Chloride Hydrochloride (1 in 500) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 100 mL of water: the pH of this solution is between 2.7 and 3.4.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(2) Sulfate ⟨*1.14*⟩—Weigh 1.5 g of Thiamine Chloride Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Nitrate—Dissolve 0.5 g of Thiamine Chloride Hydrochloride in 25 mL of water. Add 2 mL of sulfuric acid to 2 mL of this solution, shake, cool, and superimpose iron (II) sulfate TS: no dark brown ring is produced at the junction of the two layers.

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Thiamine Chloride 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) Related substances—Dissolve 0.10 g of Thiamine Chloride Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than thiamine obtained from sample solution is not larger than the peak area of thiamine 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.

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

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of thiamine obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiamine is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (30 mg, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Chloride Hydrochloride and Thiamine Chloride Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

$$\text{Amount (mg) of } C_{12}H_{17}ClN_4OS.HCl = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution methyl benzoate in methanol (1 in 50).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine 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, thiamine 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamine Chloride Hydrochloride Injection

Vitamin B_1 Hydrochloride Injection

チアミン塩化物塩酸塩注射液

Thiamine Chloride Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine Chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27).

Method of preparation Prepare as directed under Injections, with Thiamine Chloride Hydrochloride.

Description Thiamine Chloride Hydrochloride Injection is a clear, colorless liquid.

pH: 2.5 – 4.5

Identification To a volume of Thiamine Chloride Hydrochloride Injection, equivalent to 0.05 g of Thiamine Chloride Hydrochloride according to the labeled amount, add water to make 25 mL. Proceed with 5 mL of this solution as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

Bacterial endotoxins ⟨*4.01*⟩ Less than 6.0 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dilute with 0.001 mol/L hydrochloric acid TS if necessary, then measure exactly a volume of Thiamine Chloride Hydrochloride Injection, equivalent to about 20 mg of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$), and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

$$\text{Amount (mg) of thiamine chloride hydrochloride } (C_{12}H_{17}ClN_4OS.HCl) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of Thiamine Chloride Hydrochloride

RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thiamine Chloride Hydrochloride Powder

Vitamin B_1 Hydrochloride Powder

チアミン塩化物塩酸塩散

Thiamine Chloride Hydrochloride Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27).

Method of preparation Prepare as directed under Powders, with Thiamine Chloride Hydrochloride.

Identification To a portion of Thiamine Chloride Hydrochloride Powder, equivalent to 0.02 g of Thiamine Chloride Hydrochloride according to the labeled amount, add 50 mL of water and 10 mL of dilute acetic acid, shake, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

Purity Rancidity—Thiamine Chloride Hydrochloride Powder has no unpleasant or rancid odor. It is tasteless.

Assay Weigh accurately a quantity of Thiamine Chloride Hydrochloride Powder, equivalent to about 20 mg of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$), add 60 mL of 0.01 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. Shake vigorously for 10 minutes, cool, add methanol to make exactly 100 mL, and centrifuge. Pipet 25 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 50 mL of 0.01 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

Amount (mg) of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

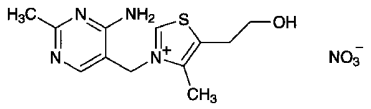
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamine Nitrate

Vitamin B_1 Nitrate

チアミン硝化物



$C_{12}H_{17}N_5O_4S$: 327.36
3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium nitrate
[*532-43-4*]

Thiamine Nitrate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{12}H_{17}N_5O_4S$.

Description Thiamine Nitrate occurs as white crystals or crystalline powder. It is odorless or a slight, characteristic odor.

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

Melting point: about 193°C (with decomposition).

Identification **(1)** Take 2-mL portions of a solution of Thiamine Nitrate (1 in 500), and add 2 to 3 drops of iodine TS: a red-brown precipitate or turbidity is produced. Upon further addition of 1 mL of 2,4,6-trinitrophenol TS, a yellow precipitate or turbidity is produced.

(2) To 1 mL of a solution of Thiamine Nitrate (1 in 500) add 1 mL of lead (II) acetate TS and 1 mL of a solution of sodium hydroxide (1 in 10), and warm: the color of the solution changes through yellow to brown, and on standing, a black-brown precipitate is produced.

(3) To 5 mL of a solution of Thiamine Nitrate (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests <*1.09*> (1) and (2) for nitrate.

pH <*2.54*> Dissolve 1.0 g of Thiamine Nitrate in 100 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity **(1)** Chloride <*1.03*>—Perform the test with 0.20 g of Thiamine Nitrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(2) Sulfate <*1.14*>—Dissolve 1.5 g of Thiamine Nitrate in 30 mL of water and 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 with 0.35 mL of 0.005 mol/L sulfuric acid VS and 2 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.011%).

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Thiamine Nitrate in 30 mL of water by warming, cool, and add 12 mL of 6 mol/L acetic acid TS and 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 (not more than 20 ppm).

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Chloride Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

Amount (mg) of $C_{12}H_{17}N_5O_4S = M_S \times Q_T/Q_S \times 0.971$

M_S: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 30°C.

Mobile phase: Dissolve 1.1 g of sodium l-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine 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, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

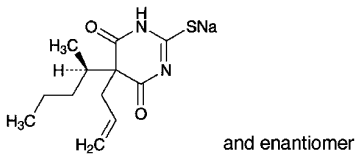
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of thiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamylal Sodium

チアミラールナトリウム



and enantiomer

$C_{12}H_{17}N_2NaO_2S$: 276.33

Monosodium 5-allyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate

[*337-47-3*]

Thiamylal Sodium contains not less than 97.5% and not more than 101.0% of $C_{12}H_{17}N_2NaO_2S$, calculated on the dried basis.

Description Thiamylal Sodium occurs as light yellow crystals or powder.

It is very soluble in water, and freely soluble in ethanol (95).

The pH of a solution of Thiamylal Sodium (1 in 10) is between 10.0 and 11.0.

It is hygroscopic.

It is gradually decomposed by light.

Its solution in ethanol (95) (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Thiamylal Sodium in ethanol (95) (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Thiamylal Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Thiamylal Sodium (1 in 10) responds to Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Clarity and color of solution—To 1.0 g of Thiamylal Sodium in a 11- to 13-mL glass-stoppered test tube add 10 mL of freshly boiled and cooled water, stopper tightly, allow to stand, and dissolve by occasional gentle shaking: the solution is clear and light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Thiamylal 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—Dissolve 0.10 g of Thiamylal Sodium in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography, develop

with a mixture of toluene, methanol and ethyl acetate (40:7:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for a night: the spot appeared around *R*f value 0.1 obtained with the sample solution is not more intense than the spot with the standard solution (2), and the spot other than the principal spot, the spot at origin and the spot mentioned above obtained with the sample solution is not more intense than the spot with the standard solution (1).

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, 105°C, 1 hour).

Assay Weigh accurately about 0.25 g of Thiamylal Sodium, dissolve in 50 mL of methanol and 5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Thiamylal RS, previously dried at 105°C for 1 hour, dissolve in 50 mL of methanol and 0.5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamylal to that of the internal standard.

$$\text{Amount (mg) of } C_{12}H_{17}N_2NaO_2S = M_S \times Q_T/Q_S \times 10 \times 1.086$$

M_S: Amount (mg) of Thiamylal RS

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column about 4 mm in inside 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 methanol and 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.6 (13:7).

Flow rate: Adjust the flow rate so that the retention time of thiamylal 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, thiamylal and the internal standard 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamylal to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamylal Sodium for Injection

注射用チアミラールナトリウム

Thiamylal Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$: 276.33).

Method of preparation Prepare as directed under Injections, with 100 parts of Thiamylal Sodium and 7 parts of Dried Sodium Carbonate in mass.

Description Thiamylal Sodium for Injection occurs as light yellow crystals, powder or masses.

It is hygroscopic.

It is gradually decomposed by light.

Identification (1) To 1.0 g of Thiamylal Sodium for Injection add 20 mL of ethanol (95), shake vigorously, and filter. Dissolve the precipitate so obtained in 1 mL of water, and add 1 mL of barium chloride TS: a white precipitate is produced. Centrifuge this solution, take off the supernatant liquid, and to the precipitate add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) To 50 mg of Thiamylal Sodium for Injection add 100 mL of ethanol (95), shake vigorously, and filter. To 3 mL of the filtrate add ethanol (95) to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Thiamylal Sodium for Injection in 40 mL of water is between 10.5 and 11.5.

Purity Related substances—To 0.10 g of Thiamylal Sodium for Injection add 10 mL of ethanol (95), shake vigorously, filter, and use the filtrate as the sample solution. Proceed as diected in the Purity (3) under Thiamylal Sodium.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.0 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Open carefully 10 containers of Thiamylal Sodium for Injection, dissolve the contents with water, wash out the inside of each container with water, combine them, and add water to make exactly *V* mL so that each mL contains about 5 mg of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$). Pipet 5 mL of this solution, and add 0.5 mL of dilute hydrochloric acid and methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Proceed the test with the sample solu-

tion as directed in the Assay under Thiamylal Sodium.

Amount (mg) of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$) in 1 container
$= M_S \times Q_T/Q_S \times V/50 \times 1.086$

M_S: Amount (mg) of Thiamylal RS

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thianthol

チアントール

Thianthol consists of dimethylthianthrene and ditoluene disulfide.

It contains not less than 23.5% and not more than 26.5% of sulfur (S: 32.07).

Description Thianthol is a yellowish, viscous liquid. It has a faint, agreeable odor.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and practically insoluble in water.

It, when cold, may separate crystals, which melt on warming.

Specific gravity d_{20}^{20}: 1.19 – 1.23

Identification To 0.1 g of Thianthol add cautiously 5 mL of sulfuric acid: a blue-purple color develops. Add 5 to 6 drops of nitric acid to the solution: the color of the solution changes to yellow-red with evolution of gas.

Purity **(1)** Acidity or alkalinity—Shake 10 g of Thianthol with 20 mL of water, allow to stand, and separate the water layer. The solution is neutral.

(2) Sulfate—To 10 mL of the water layer obtained in (1) add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg of Thianthol, and proceed as directed in the sulfur determination of Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 5 mL of diluted sodium hydroxide TS (1 in 10) and 1.0 mL of hydrogen peroxide TS as an absorbing liquid.

Containers and storage Containers—Tight containers.

Compound Thianthol and Salicylic Acid Solution

複方チアントール·サリチル酸液

Compound Thianthol and Salicylic Acid Solution contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), and not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Thianthol	200 mL
Salicylic Acid	20 g
Phenol	20 g
Olive Oil	50 mL
Ether	100 mL
Petroleum Benzin	a sufficient quantity
	To make 1000 mL

Dissolve Salicylic Acid and Phenol in Ether, add Thianthol, Olive Oil and Petroleum Benzin to this solution, mix and dissolve to make 1000 mL.

Description Compound Thianthol and Salicylic Acid Solution is a light yellow liquid, having a characteristic odor.

Identification **(1)** Place 1 mL of Compound Thianthol and Salicylic Acid Solution to a porcelain dish, and evaporate on a water bath to dryness. To the residue add cautiously 5 mL of sulfuric acid: the color of the solution changes to yellow-red with evolution of gas (thianthol).

(2) Shake 10 mL of Compound Thianthol and Salicylic Acid Solution with 10 mL of sodium hydrogen carbonate TS, and separate the water layer. To 0.5 mL of the water layer add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Wash the upper phase obtained in (2) with 10 mL of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrate TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(4) To 1 mL of Compound Thianthol and Salicylic Acid Solution add 10 mL of ethanol (95), mix, and use this solution as the sample solution. Dissolve 0.01 g each of salicylic acid, phenol and thianthol in 5 mL each of ethanol (95), and use each solution as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): three spots obtained from the sample solution and the corresponding spots of standard solutions (1), (2) and (3) show the same *R*f value. Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Assay Measure exactly 2 mL of Compound Thianthol and Salicylic Acid Solution, add exactly 10 mL of the internal standard solution, then add 70 mL of diluted methanol (1 in 2), mix well, and add diluted methanol (1 in 2) to make 100 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 0.2 g of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and diluted methanol (1 in

2) to make 100 mL, and use this solution as the standard solution. With 5 μL each of the sample solution and standard solution, perform the test as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/5$$

$$\text{Amount (mg) of phenol } (C_6H_6O) = M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/5$$

M_{Sa}: Amount (mg) of salicylic acid for assay
M_{Sb}: Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 10,000).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

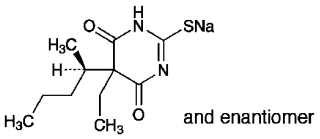
Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 25°C.

Thiopental Sodium

チオペンタールナトリウム



$C_{11}H_{17}N_2NaO_2S$: 264.32

Monosodium 5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate

[*71-73-8*]

Thiopental Sodium, when dried, contains not less than 97.0% of $C_{11}H_{17}N_2NaO_2S$.

Description Thiopental Sodium occurs as a light yellow powder. It has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Thiopental Sodium (1 in 10) is alkaline.

It is hygroscopic.

Its solution gradually decomposes on standing.

Identification **(1)** Dissolve 0.2 g of Thiopental Sodium in 5 mL of sodium hydroxide TS, and add 2 mL of lead (II) acetate TS: a white precipitate, which dissolves upon heating, is produced. Boil the solution thus obtained: a black precipitate forms gradually, and the precipitate responds to the Qualitative Tests ⟨1.09⟩ for sulfide.

(2) Dissolve 0.5 g of Thiopental Sodium in 15 mL of water, add 10 mL of dilute hydrochloric acid to produce white precipitate, and extract with four 25-mL portions of chloroform. Combine the chloroform extracts, evaporate on a water bath, and dry at 105°C for 2 hours: the residue melts ⟨2.60⟩ between 157°C and 162°C.

(3) A solution of Thiopental Sodium (1 in 10) responds to the Qualitative Tests ⟨1.09⟩ (1) and (2) for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Thiopental Sodium in 10 mL of freshly boiled and cooled water: the solution is clear and light yellow.

(2) Heavy metals ⟨1.07⟩—Dissolve 2.0 g of Thiopental Sodium in 76 mL of water, add 4 mL of dilute hydrochloric acid, shake, and filter through a glass filter (G4). To 40 mL of the filtrate add 2 mL of ammonium acetate TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare a control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 2 mL of ammonium acetate TS and water to make 50 mL (not more than 20 ppm).

(3) Neutral and basic substances—Weigh accurately about 1 g of Thiopental Sodium, dissolve in 10 mL of water and 5 mL of sodium hydroxide TS, and shake vigorously with 40 mL of chloroform. Separate the chloroform layer, wash with two 5-mL portions of water, filter, and evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour: the amount of the residue is not more than 0.50%.

(4) Related substances—Dissolve 50 mg of Thiopental Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than thiopental from the sample solution is not larger than the peak area of thiopental from the standard solution.

Operating conditions—

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

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

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time

of thiopental is about 15 minutes.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of thiopental obtained from 20 μL of this solution is equivalent to 15 to 25% of that from 20 μL of the standard solution.

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 procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

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

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Assay Weigh accurately about 0.5 g of Thiopental Sodium, 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 portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Combine the filtrate and the washings, and add 10 mL of ethanol (95). Titrate ⟨*2.50*⟩ with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light 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
= 26.43 mg of $C_{11}H_{17}N_2NaO_2S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Thiopental Sodium for Injection

注射用チオペンタールナトリウム

Thiopental Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$: 264.32).

Method of preparation Prepare as directed under Injections, with 100 parts of Thiopental Sodium and 6 parts of Dried Sodium Carbonate in mass.

Description Thiopental Sodium for Injection is a light yellow powder or mass, and has a slight, characteristic odor.

It is very soluble in water, and practically insoluble in dehydrated diethyl ether.

It is hygroscopic.

Identification **(1)** Dissolve 0.1 g of Thiopental Sodium for Injection in 10 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed. Collect the precipitate, and add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) Proceed as directed in the Identification under Thiopental Sodium.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Thiopental Sodium for Injection in 40 mL of water: the pH of this solution is between 10.2 and 11.2.

Purity Proceed as directed in the Purity under Thiopental Sodium.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.30 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take 10 samples of Thiopental Sodium for Injection, and open each container carefully. Dissolve each content with water, wash each container with water, combine the washings with the former solution, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 15 mg of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$), and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 15 mL of diluted dilute sodium hydroxide TS (1 in 100), add water to make exactly 30 mL, and use this solution as the sample solution. Separately, weigh accurately about 46 mg of thiopental for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of dilute sodium hydroxide TS, and add water to make exactly 200 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 the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 304 nm.

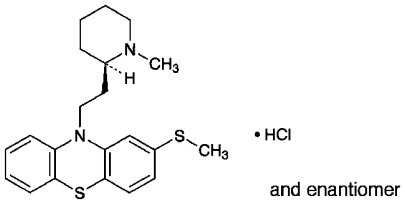
Amount (mg) of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$) in each sample of Thiopental Sodium for Injection
$= M_S \times A_T/A_S \times 300/V \times 1.091$

M_S: Amount (mg) of thiopental sodium for assay

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Thioridazine Hydrochloride

チオリダジン塩酸塩



$C_{21}H_{26}N_2S_2$.HCl: 407.04
10-{2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl}-2-methylsulfanyl-10*H*-phenothiazine monohydrochloride
[*130-61-0*]

Thioridazine Hydrochloride, when dried, contains not less than 99.0% of $C_{21}H_{26}N_2S_2$.HCl.

Description Thioridazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Thioridazine Hydrochloride (1 in 100) is between 4.2 and 5.2.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of sulfuric acid: a deep blue color develops.

(2) Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of water, and add 1 drop of cerium (IV) tetraammonium sulfate TS: a blue color develops, and the color disappears on the addition of excess of the reagent.

(3) Determine the infrared absorption spectrum of Thioridazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Thioridazine Hydrochloride (1 in 100) add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. After cooling, filter, and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*> 159 – 164°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Thioridazine 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 <*1.11*>—Prepare the test solution with 1.0 g of Thioridazine Hydrochloride, according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure under the protection from the sunlight. Dissolve 0.10 g of Thioridazine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample 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, 2-propanol and ammonia solution (28) (74:25:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

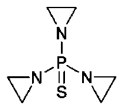
Assay Weigh accurately about 0.35 g of Thioridazine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.70 mg of $C_{21}H_{26}N_2S_2$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Thiotepa

チオテパ



$C_6H_{12}N_3PS$: 189.22
Tris(aziridin-1-yl)phosphine sulfide
[*52-24-4*]

Thiotepa, when dried, contains not less than 98.0% of $C_6H_{12}N_3PS$.

Description Thiotepa occurs as colorless or white crystals, or white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in diethyl ether.

A solution of Thiotepa (1 in 10) is neutral.

Identification **(1)** To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of hexaammonium heptamolybdate TS, and allow to stand: a dark blue color develops slowly when the solution is cold, or quickly when warm.

(2) To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of nitric acid: this solution responds to the Qualitative Tests <*1.09*> (2) for phosphate.

(3) Dissolve 0.1 g of Thiotepa in a mixture of 1 mL of lead (II) acetate TS and 10 mL of sodium hydroxide TS, and boil: the gas evolved changes moistened red litmus paper to blue, and the solution shows a grayish red color.

Melting point <*2.60*> 52 – 57°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Thiotepa in 20 mL of water: the solution is clear and col-

orless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Thiotepa 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).

(3) Arsenic <*1.11*>—Dissolve 0.20 g of Thiotepa in 5 mL of water, and add 1 mL of nitric acid and 1 mL of sulfuric acid. Take this solution, prepare the test solution according to Method 2, and perform the test (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

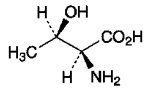
Assay Weigh accurately about 0.1 g of Thiotepa, previously dried, dissolve in 50 mL of a solution of potassium thiocyanate (3 in 20), add 25 mL of 0.05 mol/L sulfuric acid VS, exactly measured, and allow to stand for 20 minutes with occasional shaking. Titrate <*2.50*> the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS
= 6.307 mg of $C_6H_{12}N_3PS$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

L-Threonine

L-トレオニン



$C_4H_9NO_3$: 119.12
(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid
[*72-19-5*]

L-Threonine, when dried, contains not less than 98.5% of $C_4H_9NO_3$.

Description L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of L-Threonine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -26.0 – $-29.0°$ (after drying, 1.5 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.20 g of L-Threonine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

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

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Threonine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Threonine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Threonine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Threonine 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).

(6) Arsenic <*1.11*>—Dissolve 1.0 g of L-Threonine in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Threonine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 <*2.03*>. Spot 5 μL each of the sample solution and 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 evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of L-Threonine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> 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
= 11.91 mg of $C_4H_9NO_3$

Containers and storage Containers—Tight containers.

Thrombin

トロンビン

Thrombin is prepared from prothrombin obtained from blood of man or bull, through interaction with added thromboplastin in the presence of calcium ions, sterilized and lyophilized.

It contains not less than 80% and not more than 150% of the labeled Units of thrombin.

Each mg contains not less than 10 Units of thrombin.

Description Thrombin is a white to light yellow, amor-

phous substance.

Thrombin (500 Units) dissolves in 1.0 mL of isotonic sodium chloride solution clearly or with slight turbidity within 1 minute.

Loss on drying ⟨*2.41*⟩ Not more than 3% (50 mg, in vacuum, phosphorus (V) oxide, 4 hours).

Sterility ⟨*4.06*⟩ It meets the requirement.

Assay **(i)** Fibrinogen solution—Weigh accurately about 30 mg of fibrinogen, and dissolve in 3 mL of isotonic sodium chloride solution. Allow the solution to clot sufficiently with frequent shaking after the addition of about 3 Units of thrombin. Wash the precipitated clot thoroughly until the washings yield no turbidity on addition of silver nitrate TS, weigh the clot after drying at 105°C for 3 hours, and calculate the percentage of the clot in the fibrinogen. Dissolve the fibrinogen in isotonic sodium chloride solution so that the clot should be 0.20%, adjust the pH of the solution between 7.0 and 7.4 by addition of 0.05 mol/L dibasic sodium phoshate TS (or if necesary, use 0.5 mol/L disodium hydrogenphosphate TS), and dilute with isotonic sodium chloride solution to make a 0.10% solution.

(ii) Procedure—Dissolve Thrombin RS in isotonic sodium chloride solution, and prepare four kinds of standard solutions which contain 4.0, 5.0, 6.2, and 7.5 Units in 1 mL. Transfer accurately 0.10 mL each of the standard solutions maintained at a given degree ± 1°C between 20°C and 30°C to a small test tube, 10 mm in inside diameter, 100 mm in length, blow out 0.90 mL of the fibrinogen solution at the same temperature into the test tube from a pipet, start a stop watch simultaneously, shake the tube constantly, and determine the time for the first appearance of clot. Calculate the average values of five determinations for the four kinds of standard solutions, respectively. If the deviation between the maximum and the minimum values of five determinations is more than 10% of the average value, reject the whole run, and try the experiment again. The concentration of the standard solution may be changed appropriately within the range between 14 and 60 seconds of the clotting time. The determination proceeds at the same temperature described above. Next, weigh accurately the whole contents of a single container of Thrombin, dissolve it in isotonic sodium chloride solution to provide a solution which is presumed to contain about 5 Units in each mL, treat 0.10 mL of the solution with the same reagents in the same manner five times, determine the clotting times, and calculate the average value. Plot the average values of the clotting times of the four kinds of the standard solutions on a logarithmic graph, using Units as the abscissa and clotting times as the ordinate, and draw a calibration line which best fits the four plotted points. Using this line, read the Units *U* from the average value of the clotting times of the sample solution.

$$\text{Units of 1 container of Thrombin} = U \times 10 \times V$$

V: The number of mL of the volume in which the contents of 1 container of Thrombin has been dissolved

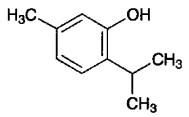
Calculate the units for 1 mg of the contents.

Containers and storage Containers—Hermetic containers.
Storage—Not exceeding 10°C.

Expiration date 36 months after preparation.

Thymol

チモール



$C_{10}H_{14}O$: 150.22
5-Methyl-2-(1-methylethyl)phenol
[*89-83-8*]

Thymol contains not less than 98.0% of $C_{10}H_{14}O$.

Description Thymol occurs as colorless crystals or white, crystalline masses. It has an aromatic odor, and has a burning taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in diethyl ether, and slightly soluble in water.

It sinks in water, but when warmed, it melts and rises to the surface of water.

Identification **(1)** To 1 mL of a solution of Thymol in acetic acid (100) (1 in 300) add 6 drops of sulfuric acid and 1 drop of nitric acid: a blue-green color develops by reflected light and a red-purple color develops by transmitted light.

(2) Dissolve 1 g of Thymol in 5 mL of a solution of sodium hydroxide (1 in 10) by heating in a water bath, and continue heating for several minutes: a light yellow-red color slowly develops. Allow this solution to stand at room temperature: the color changes to dark yellow-brown. Shake this solution with 2 to 3 drops of chloroform: a purple color gradually develops.

(3) Triturate Thymol with an equal mass of camphor or menthol: the mixture liquefies.

Melting point ⟨*2.60*⟩ 49 – 51°C

Purity **(1)** Non-volatile residue—Volatilize 2.0 g of Thymol by heating on a water bath, and dry the residue at 105°C for 2 hours: the mass is not more than 1.0 mg.

(2) Other phenols—Shake vigorously 1.0 g of Thymol with 20 mL of warm water for 1 minute, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color may develop, but no blue to purple color develops.

Assay Weigh accurately about 0.5 g of Thymol, dissolve in 10 mL of sodium hydroxide TS, and add water to make exactly 100 mL. Measure exactly 10 mL of the solution into an iodine flask, add 50 mL of water and 20 mL of dilute sulfuric acid, and cool in ice water for 30 minutes. Add exactly 20 mL of 0.05 mol/L bromine VS, stopper tightly immediately, allow to stand for 30 minutes in ice water with occasional shaking in a dark place, add 14 mL of potassium iodide TS and 5 mL of chloroform, stopper tightly, shake vigorously, and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Stopper tightly, shake vigorously near the end point, and continue the titration until the blue color in the chloroform layer disappears. Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 3.756 mg of $C_{10}H_{14}O$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Dried Thyroid

乾燥甲状腺

Dried Thyroid is the fresh thyroid gland, previously deprived of connective tissue and fat, minced, dried rapidly at a temperature not above 50°C, and powdered, or diluted with suitable diluents. It is obtained from domesticated animals that are used for food by man.

It contains not less than 0.30% and not more than 0.35% of iodine (I: 126.90) in the form of organic compounds peculiar to the thyroid gland.

Description Dried Thyroid occurs as a light yellow to grayish brown powder. It has a slight, characteristic, meat-like odor.

Identification Mount Dried Thyroid in diluted formaldehyde solution (1 in 10), stain in hematoxylin TS for 10 to 30 minutes, wash with water, soak in a mixture of 1 mL of hydrochloric acid and 99 mL of diluted ethanol (7 in 10) for 5 to 10 seconds, and again wash with water for about 1 hour. Stain in a solution of eosin Y (1 in 100) for 1 to 5 minutes, wash with water, dehydrate, and soak successively in diluted ethanol (7 in 10) for 5 to 10 seconds, in diluted ethanol (4 in 5) for 5 to 10 seconds, in diluted ethanol (9 in 10) for 1 to 2 minutes, in ethanol (95) for 1 to 5 minutes then in ethanol (99.5) for 1 to 5 minutes. Interpenetrate in xylene, seal with balsam, and examine under a microscope: epithelial nuclei forming follicles peculiar to the thyroid gland are observed.

Purity **(1)** Inorganic iodides—Mix 1.0 g of Dried Thyroid with 10 mL of a saturated solution of zinc sulfate, shake for 5 minutes, and filter. To 5 mL of the filtrate add 0.5 mL of starch TS, 4 drops of sodium nitrite TS and 4 drops of dilute sulfuric acid with thorough shaking: no blue color is produced.

(2) Fat—Extract 1.0 g of Dried Thyroid with diethyl ether for 2 hours using a Soxhlet extractor. Evaporate the diethyl ether extract, and dry the residue at 105°C to constant mass: the mass of the residue is not more than 30 mg.

Loss on drying ⟨*2.41*⟩ Not more than 6.0% (1 g, 105°C, constant mass).

Total ash ⟨*5.01*⟩ Not more than 5.0% (0.5 g).

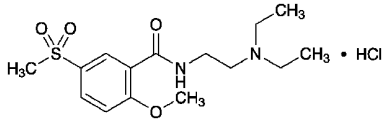
Assay Transfer about 1 g of Dried Thyroid, accurately weighed, to a crucible, add 7 g of potassium carbonate, mix carefully, and gently tap the crucible on the table to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again thoroughly by tapping. Place the crucible in a muffle furnace preheated to a temperature between 600°C and 700°C, and ignite the mixture for 25 minutes. Cool, add 20 mL of water, heat gently to boiling, and filter into a flask. To the residue add 20 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with boiling water until the filtrate measures 200 mL. Add slowly 7 mL of freshly prepared bromine TS, 40 mL of diluted phosphoric acid (1 in 2), and boil until starch iodide paper is no longer colored blue by the evolved gas. Wash down inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume at not less than 200 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate ⟨*2.50*⟩ immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.2115 mg of I

Containers and storage Containers—Tight containers.

Tiapride Hydrochloride

チアプリド塩酸塩



$C_{15}H_{24}N_2O_4S.HCl$: 364.89
N-[2-(Diethylamino)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide monohydrochloride
[*51012-33-0*]

Tiapride Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{15}H_{24}N_2O_4S.HCl$.

Description Tiapride Hydrochloride occurs as a white to slightly yellowish white crystal or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in methanol, slightly soluble in ethanol (99.5) and very slightly soluble in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Tiapride Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tiapride Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiapride Hydrochloride (1 in 20) responds to Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Tiapride 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—Dissolve 0.20 g of Tiapride Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography <2.03>. Spot rapidly 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of water, 1-butanol and acetic acid (100) (2:2:1) to a distance of about 10 cm, and air-dry, and then dry the plate at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Tiapride Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.49 mg of $C_{15}H_{24}N_2O_4S.HCl$

Containers and storage Containers—Well-closed containers.

Tiapride Hydrochloride Tablets

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Tiapride Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiapride ($C_{15}H_{24}N_2O_4S$: 328.43).

Method of preparation Prepare as directed under Tablets, with Tiapride Hydrochloride.

Identification To a quantity of powdered Tiapride Hydrochloride Tablets, equivalent to 10 mg of tiapride ($C_{15}H_{24}N_2O_4S$) according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 286 nm and 290 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiapride Hydrochloride Tablets add $V/10$ mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves until the tablet is disintegrated, and add $4V/10$ mL of methanol. To this solution add exactly $V/10$ mL of the internal standard solution, shake for 30 minutes, and add methanol to make V mL so that each mL contains about 1 mg of tiapride ($C_{15}H_{24}N_2O_4S$). Centrifuge this solution for 10 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tiapride ($C_{15}H_{24}N_2O_4S$)
$= M_S \times Q_T/Q_S \times V/100 \times 0.900$

M_S: Amount (mg) of tiapride hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tiapride Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tiapride ($C_{15}H_{24}N_2O_4S$), add about 10 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of methanol, add exactly 10 mL of the internal standard solution, shake for 30 minutes, and add methanol to make 100 mL. Centrifuge this solution and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.11 g of tiapride chloride for assay, previously dried at 105°C for 2 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of Tiapride to that of the internal standard.

Amount (mg) of tiapride ($C_{15}H_{24}N_2O_4S$)
$= M_S \times Q_T/Q_S \times 0.900$

M_S: Amount (mg) of tiapride chloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside 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: Dissolve 11.2 g of sodium perchlorate in 800 mL of water, add 5 mL of diluted perchloric acid (17 in 2000). To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tiapride 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, tiapride 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tiapride to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tiaramide Hydrochloride

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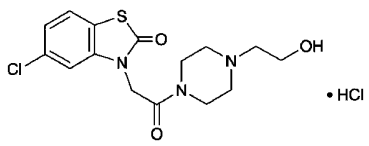

$C_{15}H_{18}ClN_3O_3S.HCl$: 392.30
5-Chloro-3-{2-[4-(2-hydroxyethyl)piperazin-1-yl]-2-oxoethyl}-1,3-benzothiazol-2(3*H*)-one monohydrochloride
[*35941-71-0*]

Tiaramide Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{18}ClN_3O_3S.HCl$.

Description Tiaramide Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Tiaramide Hydrochloride (1 in 20) is between 3.0 and 4.5.

Melting point: about 265°C (with decomposition).

Identification **(1)** Dissolve 5 mg of Tiaramide Hydrochloride in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the infrared absorption spectrum of Tiaramide Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiaramide Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Tiaramide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Tiaramide 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 <*1.11*>—Prepare the test solution with 1.0 g of Tiaramide Hydrochloride according to Method 1, and perform the test. In the procedure, add 20 mL of diluted hydrochloric acid (1 in 2) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Tiaramide Hydrochloride in 10 mL of diluted ethanol (7 in 10), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ethanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted ethanol (7 in 10) 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, air-dry the plate, and then dry at 100°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tiaramide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through purple to blue-purple (indicator: 3 drops of neutral red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.23 mg of $C_{15}H_{18}ClN_3O_3S.HCl$

Containers and storage Containers—Well-closed containers.

Tiaramide Hydrochloride Tablets

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Tiaramide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiaramide ($C_{15}H_{18}ClN_3O_3S$: 355.84).

Method of preparation Prepare as directed under Tablets, with Tiaramide Hydrochloride.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 285 nm and 289 nm, and between 292 nm and 296 nm.

(2) To a quantity of powdered Tiaramide Hydrochloride Tablets, equivalent to 0.1 g of tiaramide according to the labeled amount, add 10 mL of diluted ethanol (7 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.11 g of tiaramide hydrochloride for assay in 10 mL of diluted ethanol (7 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butamol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly Dragendorff's TS for spraying followed by diluted nitric acid (1 in 50) on the plate: the principal spot obtained with the sample solution and the spot with the standard solution are yellow-red in color and have the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test accord-

ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiaramide Hydrochloride Tablets add a volume of 0.1 mol/L hydrochloric acid TS, equivalent to 3/5 volume of V mL which makes a solution so that each mL contains about 1 mg of tiaramide ($C_{15}H_{18}ClN_3O_3S$), shake for 60 minutes, then add 0.1 mol/L hydrochloric acid TS to make exactly V mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS 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 sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of tiaramide ($C_{15}H_{18}ClN_3O_3S$)
$= M_S \times A_T/A_S \times V/50 \times 0.907$

M_S: Amount (mg) of tiaramide hydrochloride for assay

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates of a 50-mg tablet in 15 minutes and of a 100-mg tablet in 30 minutes are not less than 80%.

Start the test with 1 tablet of Tiaramide Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 56 μg of tiaramide ($C_{15}H_{18}ClN_3O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 294 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of tiaramide ($C_{15}H_{18}ClN_3O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 360 \times 0.907$

M_S: Amount (mg) of tiaramide hydrochloride for assay
C: Labeled amount (mg) of tiaramide ($C_{15}H_{18}ClN_3O_3S$) in 1 tablet

Assay Weigh accurately the mass of more than 20 Tiaramide Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tiaramide ($C_{15}H_{18}ClN_3O_3S$), add 60 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS 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, of the sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of tiaramide ($C_{15}H_{18}ClN_3O_3S$)
$= M_S \times A_T/A_S \times 0.907$

M_S: Amount (mg) of tiaramide hydrochloride for assay

Containers and storage Containers—Tight containers.

Ticlopidine Hydrochloride

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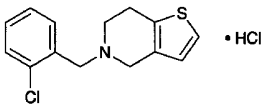

$C_{14}H_{14}ClNS.HCl$: 300.25
5-(2-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine monohydrochloride
[*53885-35-1*]

Ticlopidine Hydrochloride contains not less than 99.0% of $C_{14}H_{14}ClNS.HCl$, calculated on the anhydrous basis.

Description Ticlopidine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in water and in methanol, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Determine the infrared absorption spectrum of Ticlopidine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ticlopidine Hydrochloride (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ticlopidine Hydrochloride according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ticlopidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.5 g of Ticlopidine Hydrochloride in 20 mL of a solution of hydrochloric acid in methanol (1 in 20,000), and use this solution as the sample solution. To exactly 5 mL of the sample solution add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, pipet 1 mL of the sample solution, add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution (1) on a plate of silica gel for thin-layer chromatography (Plate 1), and

spot 10 μL each of the sample solution and standard solution (2) on another plate of silica gel for thin-layer chromatography (Plate 2). Develop the plates with an upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 15 cm, and air-dry the plates. Spray evenly a solution of ninhydrin in acetone (1 in 50) on Plate 1, and heat at 100°C for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Allow Plate 2 to stand in an iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

(4) Formaldehyde—Dissolve 0.80 g of Ticlopidine Hydrochloride in 19.0 mL of water, add 1.0 mL of 4 mol/L sodium hydroxide TS, shake well, centrifuge, and filter the supernatant liquid. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, mix, and warm at 40°C for 40 minutes: the solution has no more color than the following control solution.

Control solution: Weigh exactly 0.54 g of formaldehyde solution, and add water to make exactly 1000 mL. To exactly 10 mL of this solution add water to make exactly 1000 mL. Prepare before use. To 8.0 mL of this solution add water to make 20.0 mL, and filter. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, and proceed in the same manner.

Water ⟨*2.48*⟩ Not more than 1.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ticlopidine Hydrochloride, dissolve in 20 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ 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
= 30.03 mg of $C_{14}H_{14}ClNS.HCl$

Containers and storage Containers—Well-closed containers.

Timepidium Bromide Hydrate

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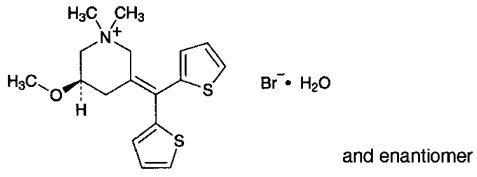

and enantiomer

$C_{17}H_{22}BrNOS_2.H_2O$: 418.41
(5*RS*)-3-(Dithien-2-ylmethylene)-5-methoxy-1,1-dimethylpiperidinium bromide monohydrate
[*35035-05-3*, anhydride]

Timepidium Bromide Hydrate contains not less than 98.5% of timepidium bromide ($C_{17}H_{22}BrNOS_2$: 400.40), calculated on the anhydrous basis.

Description Timepidium Bromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (99.5), sparingly soluble in water and in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Timepidium Bromide Hydrate in freshly boiled and cooled water (1 in 100) is between 5.3 and 6.3.

A solution of Timepidium Bromide Hydrate in methanol (1 in 20) shows no optical rotation.

Identification **(1)** To 1 mL of a solution of Timepidium Bromide Hydrate (1 in 100) add 1 mL of ninhydrin-sulfuric acid TS: a red purple color develops.

(2) Determine the absorption spectrum of a solution of Timepidium Bromide Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Timepidium Bromide Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhitit similar intensities of absorption at the same wave numbers.

(4) A solution of Timepidium Bromide Hydrate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (1) for Bromide.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Timepidium Bromide 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 0.10 g of Timepidium Bromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample 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, methanol, water, acetic acid (100) and ethyl acetate (5:4:1:1:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ 3.5 – 5.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Timepidium Bromide Hydrate, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), and titrate ⟨*2.50*⟩ 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
= 40.04 mg of $C_{17}H_{22}BrNOS_2$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Timolol Maleate

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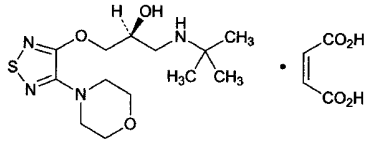


$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$: 432.49
(2*S*)-1-[(1,1-Dimethylethyl)amino]-3-(4-morpholin-4-yl-1,2,5-thiadiazol-3-yloxy)propan-2-ol monomaleate
[*26921-17-5*]

Timolol Maleate, when dried, contains not less than 98.0% and not more than 101.0% of $C_{13}H_{24}N_4O_3S.C_4H_4O_4$.

Description Timolol Maleate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 197°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Timolol Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Timolol Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Timolol Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -5.7 – $-6.2°$ (after drying, 1.25 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Timolol Maleate in 20 mL of water is between 3.8 and 4.3.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Timolol Maleate in 20 mL of water: the solution is clear, and its absorbance at 440 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.05.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Timolol Maleate 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 30 mg of Timolol Maleate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than timolol and maleic acid obtained from sample solution is not larger than 1/5 times the peak area of timolol from the standard solution, and the total area of the peaks other than the peak of timolol and maleic acid is not larger than 1/2 times the peak area of timolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside 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 40°C.

Mobile phase: Dissolve 1.9 g of sodium 1-hexanesulfonate in 1800 mL of water, add 6.0 mL of triethylamine and 8.0 mL of formic acid, adjust to pH 3.0 with formic acid, and add water to make 2000 mL. To 1400 mL of this solution add 500 mL of methanol and 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of timolol is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of timolol beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make 10 mL. Confirm that the peak area of timolol obtained from 25 μL of this solution is equivalent to 7 to 13% of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of timolol are not less than 1500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of timolol is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 100°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

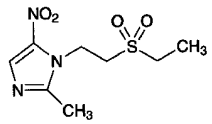
Assay Weigh accurately about 0.8 g of Timolol Maleate, previously dried, dissolve in 90 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.25 mg of $C_{13}H_{24}N_4O_3S.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Tinidazole

チニダゾール



$C_8H_{13}N_3O_4S$: 247.27
1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole
[*19387-91-8*]

Tinidazole, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{13}N_3O_4S$.

Description Tinidazole occurs as a light yellow, crystalline powder.

It is soluble in acetic anhydride and in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Tinidazole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tinidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 125 – 129°C

Purity **(1)** Sulfate <*1.14*>—To 2.0 g of Tinidazole add 100 mL of water, boil for 5 minutes, cool, add water to make 100 mL, and filter. Take 25 mL of the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.043%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tinidazole 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 <*1.11*>—Prepare the test solution with 2.0 g of Tinidazole according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Tinidazole in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone 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 <*2.03*>. Spot 10 μL each of the sample 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 and diethylamine (19:1) to a distance of about 10 cm, air-dry the plate, heat at 100°C for 5 minute, and cool. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

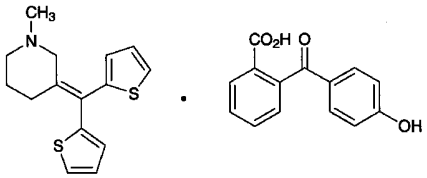
Assay Weigh accurately about 0.35 g of Tinidazole, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.73 mg of $C_8H_{13}N_3O_4S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Tipepidine Hibenzate

チペピジンヒベンズ酸塩



$C_{15}H_{17}NS_2.C_{14}H_{10}O_4$: 517.66
3-(Dithien-2-ylmethylene)-1-methylpiperidine mono[2-(4-hydroxybenzoyl)benzoate]
[*31139-87-4*]

Tipepidine Hibenzate, when dried, contains not less than 98.5% of $C_{15}H_{17}NS_2.C_{14}H_{10}O_4$.

Description Tipepidine Hibenzate occurs as a white to light yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 0.01 g of Tipepidine Hibenzate in 5 mL of sulfuric acid: an orange-red color develops.

(2) Dissolve 0.3 g of Tipepidine Hibenzate in 10 mL of sodium hydroxide TS and 5 mL of water, and extract with two 20-mL portions of chloroform. Wash the chloroform extracts with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, and dissolve the residue in 0.5 mL of 1 mol/L hydrochloric acid TS and 5 mL of water. To 2 mL of this solution add 5 mL of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Tipepidine Hibenzate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tipepidine Hibenzate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 189 – 193°C

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Tipepidine Hibenzate in 10 mL of acetic acid (100): the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: its absorbance at 400 nm is not more than 0.16.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Tipepidine Hibenzate 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 <*1.11*>—Prepare the test solution with 1.0 g of Tipepidine Hibenzate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—(i) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than hibenzic acid and tipepidine from the sample solution is not larger than the peak area of the tipepidine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 50°C.

Mobile phase: A mixture of a solution of ammonium acetate (1 in 100) and tetrahydrofuran (32:13).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 12 minutes.

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with the resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 1.5%.

(ii) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than hibenzic acid and tipepidine from the sample solution is not larger than 1/2 times the peak area of the tipepidine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 a solution of ammonium acetate (1 in 500) (13:7).

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

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 12 mg of Tipepidine Hibenzate and 4 mg of xanthene in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and xanthene are eluted in this order with the resolution between the peaks of tipepidine and xanthene being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 3.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 60°C, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tipepidine Hibenzate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (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
= 51.77 mg of $C_{15}H_{17}NS_2.C_{14}H_{10}O_4$

Containers and storage Containers— Well-closed containers.

Storagle—Light-resistant.

Tipepidine Hibenzate Tablets

チペピジンヒベンズ酸塩錠

Tipepidine Hibenzate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$: 517.66).

Method of preparation Prepare as directed under Tablets, with Tipepidine Hibenzate.

Identification **(1)** To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 44 mg of Tipepidine Hibenzate according to the labeled amount, add 5 mL of water, shake for 1 minute, add 10 mL of sodium hydroxide TS, and extract with two 20-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, dissolve the residue in 0.2 mL of 1 mol/L hydrochloric acid TS and 2 mL of water, and add 5 mL of Reinecke salt TS: a light red precipitate is formed.

(2) To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 11 mg of Tipepidine Hibenzate according to the labeled amount, add 30 mL of ethanol (99.5), and warm for 10 minutes with occasional shaking. After cooling, add ethanol (99.5) to make 50 mL, and filter. To 1 mL of the filtrate add ethanol (99.5) to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 282 nm and 286 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tipepidine Hibenzate Tablets add 5 mL of diluted acetic acid (100) (1 in 2) and 15 mL of methanol per 11 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and warm for 15 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly V mL so that each mL contains about 0.44 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of tipepidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Tipepidine Hibenzate Tablets is not less than 80%.

Start the test with 1 tablet of Tipepidine Hibenzate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, and dissolve in 80 mL of diluted ethanol (3 in 4) by warming occasionally. After cooling, add diluted ethanol (3 in 4) to make exactly 100 mL, then pipet 20 mL of this solution, add water to make exactly 900 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 286 nm, and A_{T2} and A_{S2}, at 360 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$)
$= M_S \times (A_{T1} - A_{T2}/A_{S1} - A_{S2}) \times 1/C \times 20$

M_S: Amount (mg) of tipepidine hibenzate for assay
C: Labeled amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Tipepidine Hibenzate Tablets. Weigh accurately a portion of the powder, equivalent to about 22 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), add 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and warm for 10 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, dissolve in 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 25 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tipepidine to that of the internal standard, respectively.

Amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of tipepidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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 a solution of sodium lauryl sulfate in diluted phosphoric acid (1 in 1000) (1 in 500), acetonitrile and 2-propanol (3:2:1).

Flow rate: Adjust the flow rate so that the retention time of tipepidine 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, tipepidine 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tipepidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Titanium Oxide

酸化チタン

TiO_2: 79.87

Titanium Oxide, when dried, contains not less than 98.5% of TiO_2.

Description Titanium Oxide occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It dissolves in hot sulfuric acid and in hydrofluoric acid, and does not dissolve in hydrochloric acid, in nitric acid and in dilute sulfuric acid.

When fused by heating with potassium hydrogen sulfate, with potassium hydroxide, or with potassium carbonate, it changes to soluble salts.

Shake 1 g of Titanium Oxide with 10 mL of water: the mixture is neutral.

Identification Heat 0.5 g of Titanium Oxide with 5 mL of sulfuric acid until white fumes are evolved, cool, add cautiously water to make 100 mL, and filter. To 5 mL of the filtrate add 2 to 3 drops of hydrogen peroxide TS: a yellow-red color develops.

Purity **(1)** Lead—Place 1.0 g of Titanium Oxide in a platinum crucible, add 10.0 g of potassium hydrogen sulfate, heat gently with caution at the beginning, then raise the temperature gradually, and heat strongly with occasional shaking until the contents fuse to yield a clear liquid. Cool, add 30 mL of a solution of diammonium hydrogen citrate (9 in 20) and 50 mL of water, dissolve by heating on a water bath, cool, add water to make 100 mL, and use this solution as the sample stock solution. Take 25 mL of the sample stock solution to a separator, add 10 mL of a solution of ammonium sulfate (2 in 5) and 5 drops of thymol blue TS, neutralize with ammonia TS, and add 2.5 mL of ammonia TS. To this solution add exactly 20 mL of a solution of dithizone in *n*-butyl acetate (1 in 500), shake for 10 minutes, and use this *n*-butyl acetate solution as the sample solution. Separately, place 6.0 mL of Standard Lead Solution in a platinum crucible, proceed as directed in the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions: the absorbance of the sample solution is smaller than that of the standard solution (not more than 60 ppm).

Gas: Combustible gas—Acetylene gas or hydrogen gas.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.3 nm.

(2) Arsenic <*1.11*>—Perform the test with 20 mL of the sample stock solution obtained in (1) as the test solution: the color is not deeper than the following color standard.

Color standard: Proceed in the same manner without Titanium Oxide, transfer 20 mL of the obtained solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 650°C to constant mass: the mass of the residue is not more than 5.0 mg.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

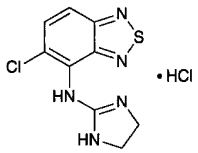
Assay Weigh accurately about 0.2 g of Titanium Oxide, previously dried, transfer to a crucible, and add 3 g of potassium disulfate. Cover, and heat gently at first, gradually raise the temperature, and then heat the fused contents for 30 minutes. Continue heating for 30 minutes at a higher temperature to make the fused mixture a deep yellow-red, almost clear liquid. Cool, transfer the contents of the crucible to a 250-mL beaker, wash the crucible with a mixture of 75 mL of water and 2.5 mL of sulfuric acid into the beaker, and heat on a water bath until the solution becomes almost clear. Dissolve 2 g of L-tartaric acid in the solution, add 2 to 3 drops of bromothymol blue TS, neutralize with ammonia TS, and acidify with 1 to 2 mL of diluted sulfuric acid (1 in 2). Pass hydrogen sulfide sufficiently through the solution, add 30 mL of ammonia TS, again saturate the solution with hydrogen sulfide, allow to stand for 10 minutes, and filter. Wash the precipitate on the filter paper with ten 25-mL portions of a mixture of ammonium L-tartrate solution (1 in 100) and ammonium sulfide TS (9:1). When the precipitate is filtered and washed, prevent iron (II) sulfide from oxidation by filling the solution on the filter paper. Combine the filtrate and the washings, add 40 mL of diluted sulfuric acid (1 in 2), and boil to expel hydrogen sulfide. Cool, and dilute with water to make 400 mL. Add gradually 40 mL of cupferron TS to the solution with stirring, and allow to stand. After sedimentation of a yellow precipitate, add again cupferron TS until a white precipitate is produced. Filter by slight suction using quantitative filter paper, wash with twenty portions of diluted hydrochloric acid (1 in 10), and remove water by stronger suction at the last washing. Dry the precipitate together with the filter paper at 70°C, transfer to a tared crucible, and heat very gently at first, and raise the temperature gradually after smoke stops evolving. Heat strongly between 900°C and 950°C to constant mass, cool, and weigh as titanium oxide (TiO_2).

Containers and storage Containers—Well-closed contain-

ers.

Tizanidine Hydrochloride

チザニジン塩酸塩



$C_9H_8ClN_5S.HCl$: 290.17
5-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazole-4-amine monohydrochloride
[*64461-82-1*]

Tizanidine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_8ClN_5S.HCl$.

Description Tizanidine Hydrochloride occurs as a white to light yellowish white crystalline powder.

It is soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

Melting point: about 290°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Tizanidine Hydrochloride in diluted 1 mol/L ammonia TS (1 in 10) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tizanidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tizanidine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Tizanidine Hydrochloride 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).

(2) Related substances—Dissolve 60 mg of Tizanidine Hydrochloride in 10 mL of a mixture of water and acetonitrile (17:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (17:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tizanidine with the sample solution is not larger than 1/5 times the peak area of tizanidine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm for about 3 minutes after sample injection and 318 nm subsequently).

Column: A stainless steel column 4.6 mm in inside 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 A: A mixture of water and formic acid (200:1), adjusted to pH 8.5 with ammonia water (28).

Mobile phase B: A mixture of acetonitrile and the mobile phase A (4:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	81 → 68	19 → 32
10 – 13	68	32
13 – 26	68 → 10	32 → 90
26 – 28	10	90

Flow rate: Adjust the flow rate so that the retention time of tizanidine is about 7 minute.

Time span of measurement: About 4 times as long as the retention time of tizanidine beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of water and acetonitrile (17:3) to make exactly 10 mL. Confirm that the peak area of tizanidine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Tizanidine Hydrochloride and *p*-toluenesulfonic acid monohydrate in 100 mL of the mixture of water and acetonitrile (17:3). When the procedure is run with 10 μL of this solution under the above operating conditions, *p*-toluenesulfonic acid and tizanidine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tizanidine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

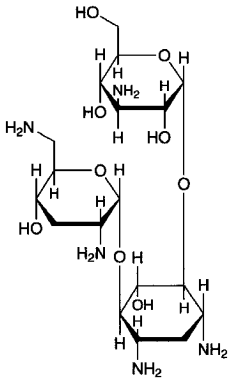
Assay Weigh accurately about 0.2 g of Tizanidine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) with the aid of warming. After cooling, titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.02 mg of $C_9H_8ClN_5S.HCl$

Containers and storage Containers—Well-closed containers.

Tobramycin

トブラマイシン



$C_{18}H_{37}N_5O_9$: 467.51
3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine
[*32986-56-4*]

Tobramycin is an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces tenebrarius*.

It contains not less than 900 μg (potency) and not more than 1060 μg (potency) per mg, calculated on the anhydrous basis. The potency of Tobramycin is expressed as mass (potency) of tobramycin ($C_{18}H_{37}N_5O_9$).

Description Tobramycin occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Determine the ^1H spectrum of a solution of Tobramycin in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ 5.1 ppm, a multiple signal B between δ 2.6 ppm and δ 4.0 ppm, and a multiple signal C between δ 1.0 ppm and δ 2.1 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 1:8:2.

(2) Dissolve 10 mg each of Tobramycin and Tobramycin RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 4 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia TS, 1-butanol and methanol (5:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100°C for 5 minutes: the *R*f values of the principal spots obtained from the sample solution and the standard solution are the same.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +138 – +148° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.10 g of Tobramycin in 10 mL of water is between 9.5 and 11.5.

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tobramycin 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) Related substances—Dissolve 80 mg of Tobramycin in 10 mL of diluted ammonia solution (28) (1 in 250), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia solution (28) (1 in 250) 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 <*2.03*>. Spot 5 μL of the sample 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 (95) and 2-butanone (1:1:1) to a distance of about 10 cm, air-dry the plate, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 11.0% (0.1 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.

Residue on ignition <*2.44*> Not more than 1.0% (0.5 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Tobramycin RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this 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 before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Tobramycin, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 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 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Tobramycin Injection

トブラマイシン注射液

Tobramycin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.51).

Method of preparation Prepare as directed under Injections, with Tobramycin.

Description Tobramycin Injection occurs as a colorless or very pale yellow clear liquid.

Identification To a volume of Tobramycin Injection, equivalent to 10 mg (potency) of Tobramycin according to the labeled amount, add water to make 1 mL, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Tobramycin RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Tobramycin.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ 5.0 – 7.0

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Tobramycin.

(ii) Sample solutions—To exactly 5 mL of Tobramycin Injection add 0.1 mol/L phosphate buffer solution, pH 8.0 so that each mL contains 1 mg (potency) of Tobramycin. 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 8 μg (potency) and 2 μg (potency), and use these solutions as the concentration sample solution high and the low concentration sample solution, respectively.

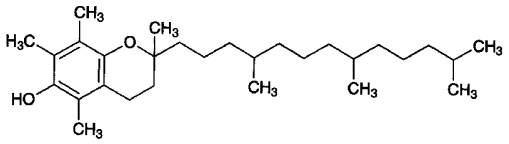
Containers and storage Containers—Hermetic containers.

Tocopherol

Vitamin E

dl-α-Tocopherol

トコフェロール



$C_{29}H_{50}O_2$: 430.71

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol

[*10191-41-0*]

Tocopherol contains not less than 96.0% and not more than 102.0% of $C_{29}H_{50}O_2$.

Description Tocopherol is a clear, yellow to red-brown, viscous liquid. It is odorless.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether and with vegetable oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is oxidized by air and light, and acquires a dark red color.

Identification **(1)** Dissolve 0.01 g of Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Determine the infrared absorption spectrum of Tocopherol as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (292 nm): 71.0 – 76.0 (10 mg, ethanol (99.5), 200 mL).

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.503 – 1.507

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.947 – 0.955

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Tocopherol in 10 mL of ethanol (99.5): the solution is clear and has no more color than Matching Fluid C.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Tocopherol 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).

Assay Dissolve about 50 mg each of Tocopherol and Tocopherol RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak heights, H_T and H_S, of tocopherol in the sample solution and standard solution.

$$\text{Amount (mg) of } C_{29}H_{50}O_2 = M_S \times H_T/H_S$$

M_S: Amount (mg) of Tocopherol RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

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

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

Mobile phase: A mixture of methanol and water (49:1).

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

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol is not more than 0.8%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and well-filled, or under nitrogen atmosphere.

Tocopherol Acetate

Vitamin E Acetate

dl-α-Tocopherol Acetate

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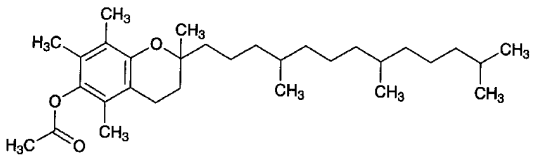

$C_{31}H_{52}O_3$: 472.74

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate

[*7695-91-2*]

Tocopherol Acetate contains not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Description Tocopherol Acetate is a clear, colorless or yellow, viscous and odorless liquid.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether, with hexane and with fixed oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is affected by air and light.

Identification **(1)** Dissolve 0.05 g of Tocopherol Acetate in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color is produced.

(2) Determine the infrared absorption spectrum of Tocopherol Acetate as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (284 nm): 41.0 – 45.0 (10 mg, ethanol (99.5), 100 mL).

Refractive index <*2.45*> n_D^{20}: 1.494 – 1.499

Specific gravity <*2.56*> d_{20}^{20}: 0.952 – 0.966

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Tocopherol Acetate in 10 mL of ethanol (99.5): the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Iron (III) Chloride CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Heavy metals <*1.07*>—Carbonize 1.0 g of Tocopherol Acetate by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (20 ppm).

(3) α-Tocopherol—Dissolve 0.10 g of Tocopherol Acetate in exactly 10 mL of hexane, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in hexane to make exactly 100 mL. Pipet 1 mL of this solution, add hexane 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α,α′-dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spot from the sample solution corresponding to that from the standard solution is not larger and not more intense than the spot from the standard solution.

Assay Dissolve 50 mg each of Tocopherol Acetate and Tocopherol Acetate RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak heights, H_T and H_S, of tocopherol acetate in the sample solution and the standard solution, respectively.

$$\text{Amount (mg) of } C_{31}H_{52}O_3 = M_S \times H_T/H_S$$

M_S: Amount (mg) of Tocopherol Acetate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

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

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

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol acetate is about 12 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol acetate is not more than 0.8%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tocopherol Calcium Succinate

Vitamin E Calcium Succinate

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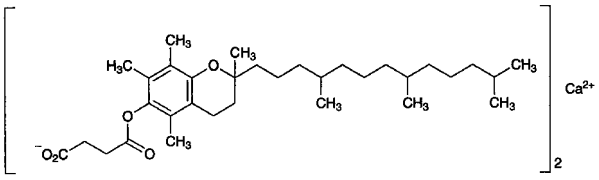

$C_{66}H_{106}CaO_{10}$: 1099.62

Monocalcium bis{3-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxycarbonyl]propanoate}

[*14638-18-7*]

Tocopherol Calcium Succinate, when dried, contains not less than 96.0% and not more than 102.0% of $C_{66}H_{106}CaO_{10}$.

Description Tocopherol Calcium Succinate occurs as a white to yellowish white powder. It is odorless.

It is freely soluble in chloroform and in carbon tetrachloride, and practically insoluble in water, in ethanol (95) and in acetone.

Shake 1 g of Tocopherol Calcium Succinate with 7 mL of acetic acid (100): it dissolves, and produces a turbidity after being allowed to stand for a while.

It dissolves in acetic acid (100).

It is optically inactive.

Identification **(1)** Dissolve 0.05 g of Tocopherol Calcium Succinate in 1 mL of aetic acid (100), add 9 mL of ethanol (99.5), and mix. To this solution add 2 mL of fuming nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Dissolve 0.08 g of Tocopherol Calcium Succinate, previously dried, in 0.2 mL of carbon tetrachloride. Determine the infrared absorption spectrum of the solution as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 5 g of Tocopherol Calcium Succinate in 30 mL of chloroform, add 10 mL of hydrochloric acid, shake for 10 minutes, then draw off the water layer, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for calcium salt.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (286 nm): 36.0 – 40.0 (10 mg, chloroform, 100 mL).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Tocopherol Calcium Succinate in 10 mL of chloroform: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Iron (III) Chloride CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Alkalinity—To 0.20 g of Tocopherol Calcium Succinate add 10 mL of diethyl ether, 2 mL of water, 1 drop of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS, and shake: no red color develops in the water layer.

(3) Chloride <*1.03*>—Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of diethyl ether, shake thoroughly, and collect the water layer. To the diethyl ether layer add 10 mL of water, shake, and collect the water layer. Combine the water layers, 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 in the same manner using 0.60 mL of 0.01 mol/L hydrochloric acid VS in place of Tocopherol Calcium Succinate (not more than 0.212%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Tocopherol Calcium Succinate 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).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Tocopherol Calcium Succinate according to Method 3, and perform the test (not more than 2 ppm).

(6) α-Tocopherol—Dissolve 0.10 g of Tocopherol Calcium Succinate in exactly 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α-α'-dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spots from the sample solution corresponding to the spots from the standard solution is not larger than and not more intense than the spots from the standard solution.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Assay Weigh accurately about 50 mg each of Tocopherol Calcium Succinate and Tocopherol Succinate RS, previously dried, dissolve in a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Pipet exactly 20 μL each of the sample solution and standard solution, and perform the test as directed under Liquid

Chromatography <2.01> according to the following operating conditions. Determine the peak heights, H_T and H_S, of tocopherol succinate in these solutions, respectively.

Amount (mg) $C_{66}H_{106}CaO_{10} = M_S \times H_T/H_S \times 1.036$

M_S: Amount (mg) of Tocopherol Succinate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to 10 μL in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol, water and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol succinate is about 8 minutes.

Selection of column: Dissolve 0.05 g each of tocopherol succinate and tocopherol in 50 mL of a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1). Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of tocopherol succinate and tocopherol in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Repeat the test 5 times with 20 μL of the standard solution under the above operating conditions: the relative standard deviation of the peak height of tocopherol succinate is not more than 0.8%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tocopherol Nicotinate

Vitamin E Nicotinate

dl-α-Tocopherol Nicotinate

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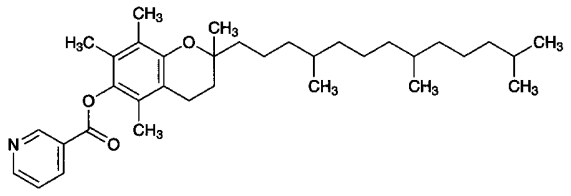


$C_{35}H_{53}NO_3$: 535.80

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl nicotinate

[*51898-34-1*]

Tocopherol Nicotinate contains not less than 96.0% of $C_{35}H_{53}NO_3$.

Description Tocopherol Nicotinate occurs as a yellow to orange-yellow liquid or solid.

It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 10) shows no optical rotation.

It is affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tocopherol Nicotinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Tocopherol Nicotinate, if necessary melt by warming, as directed in the liquid film method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Nicotinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol Nicotinate 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 <1.11>—Prepare the test solution with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tocopherol Nicotinate in 50 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 7 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 tocopherol nicotinate from the sample solution is not larger than the peak area of tocopherol nicotinate from the standard solution, and the area of a peak which has a retention time 0.8 to 0.9 times that of tocopherol nicotinate from the sample solution is not larger than 4/7 times the peak area of tocopherol nicotinate from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of methanol and water (19:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 20 minutes.

Time span of measurement: About 1.5 times as long as the retention time of tocopherol nicotinate beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of tocopherol nicotinate obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above operating conditions, tocopherol and tocopherol nicotinate 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Tocopherol Nicotinate and Tocopherol Nicotinate RS, dissolve each in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of tocopherol nicotinate of these solutions.

$$\text{Amount (mg) of } C_{35}H_{53}NO_3 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Tocopherol Nicotinate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 264 nm).

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: Methanol.

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

System suitability—

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 5 μL of this solution under the above operating conditions, tocopherol and tocopherol nicotinate 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 of the standard solution under the above operating conditions: the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 0.8%

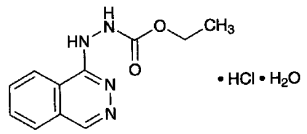
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Todralazine Hydrochloride Hydrate

Ecarazine Hydrochloride

トドララジン塩酸塩水和物



$C_{11}H_{12}N_4O_2.HCl.H_2O$: 286.71

Ethyl 2-(phthalazin-1-yl)hydrazinecarboxylate monohydrochloride monohydrate

[*3778-76-5*, anhydride]

Todralazine Hydrochloride Hydrate contains not less than 98.5% of todralazine hydrochloride ($C_{11}H_{12}N_4O_2.HCl$: 268.70), calculated on the anhydrous basis.

Description Todralazine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a slight, characteristic odor, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Todralazine Hydrochloride Hydrate (1 in 200) is between 3.0 and 4.0.

Identification **(1)** To 2 mL of a solution of Todralazine Hydrochloride Hydrate (1 in 200) add 5 mL of silver nitrate-ammonia TS: the solution becomes turbid, and a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Todralazine Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Todralazine Hydrochloride Hydrate as directed in the potassium chloride disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Todralazine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (1) for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 0.30 g of Todralazine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate <*1.14*>—Proceed the test with 2.0 g of Todralazine Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.012%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Todralazine 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).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Todralazine Hydrochloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Todralazine Hydrochloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 todralazine from the sample solution is not larger than the peak area of todralazine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 3.9 mm in inside 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: Dissolve 1.10 g of sodium 1-heptane sulfonate in 1000 mL of diluted methanol (2 in 5). Adjust the pH of the solution to between 3.0 and 3.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of todralazine is about 8 minutes.

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

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of todralazine obtained from 10 μL of this solution is equivalent to 15 to 25% of that from 10 μL of the standard solution.

System performance: Dissolve 5 mg each of Todralazine Hydrochloride Hydrate and potassium biphthalate in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, phthalic acid and todralazine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of todralazine is not more than 2.0%.

Water ⟨*2.48*⟩ 6.0 – 7.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

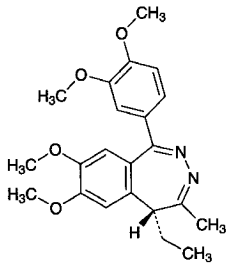
Assay Weigh accurately about 0.4 g of Todralazine Hydrochloride, dissolve in 5 mL of formic acid, add 70 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.87 mg of $C_{11}H_{12}N_4O_2.HCl$

Containers and storage Containers—Tight containers.

Tofisopam

トフィソパム



and enantiomer

$C_{22}H_{26}N_2O_4$: 382.45
(5*RS*)-1-(3,4-Dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5*H*-2,3-benzodiazepine
[*22345-47-7*]

Tofisopam, when dried, contains not less than 98.0% of $C_{22}H_{26}N_2O_4$.

Description Tofisopam occurs as a pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Tofisopam in ethanol (95) (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Tofisopam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tofisopam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 155 – 159°C

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Tofisopam 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 ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Tofisopam according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tofisopam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 25 mL, pipet 1 mL of this solution, add acetone 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 ⟨*2.03*⟩. Spot 10 μL each of the sample 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, methanol and formic acid (24:12:2:1) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

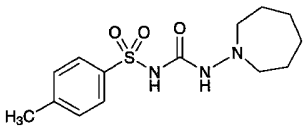
Assay Weigh accurately about 0.2 g of Tofisopam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.25 mg of $C_{22}H_{26}N_2O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Tolazamide

トラザミド



$C_{14}H_{21}N_3O_3S$: 311.40
N-(Azepan-1-ylcarbamoyl)-
4-methylbenzenesulfonamide
[*1156-19-0*]

Tolazamide, when dried, contains not less than 97.5% and not more than 102.0% of $C_{14}H_{21}N_3O_3S$.

Description Tolazamide occurs as a white to pale yellow, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetone, slightly soluble in ethanol (95) and in *n*-butylamine, and practically insoluble in water and in diethyl ether.

Melting point: about 168°C (with decomposition).

Identification **(1)** Dissolve 0.02 g of Tolazamide in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Shake well this solution with 5 mL of chloroform, and allow to stand: a green color develops in the chloroform layer.

(2) Determine the absorption spectrum of a solution of Tolazamide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolazamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolazamide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolazamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Tolazamide 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 <*1.11*>—Prepare the test solution with 1.0 g of Tolazamide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Tolazamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of *p*-toluenesulfonamide in acetone to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample 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, methanol, cyclohexane and diluted ammonia solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution corresponding 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 principal and above spots from the sample solution are not more intense than the spot from the standard solution (1).

(4) *N*-Aminohexamethyleneimine—To 0.50 g of Tolazamide add 2.0 mL of acetone, stopper the flask tightly, shake vigorously for 15 minutes. Add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, allow to stand for 15 minutes, and filter. To the filtrate add 1.0 mL of trisodium ferrous pentacyanoamine TS, and shake: the color developing within 30 minutes is not deeper than that of the following control solution.

Control solution: Dissolve 0.125 g of *N*-aminohexamethyleneimine in acetone to make exactly 100 mL. Pipet 1 mL of this solution, and add acetone to make exactly 100 mL. To 2.0 mL of this solution add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, and proceed in the same manner.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Tolazamide and Tolazamide RS, previously dried, dissolve each in exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tolazamide to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{14}H_{21}N_3O_3S = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Tolazamide RS

Internal standard solution—A solution of tolbutamide in ethanol-free chloroform (3 in 2000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 30 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 hexane, water-saturated hexane, tetrahydrofuran, ethanol (95) and acetic acid (100) (475:475:20:15:9).

Flow rate: Adjust the flow rate so that the retention time of tolazamide 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, the internal standard and tolazamide are eluted in this order with the resolution between these peaks being not

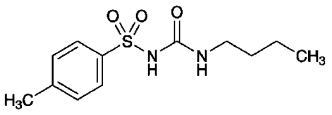
less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tolazamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tolbutamide

トルブタミド



$C_{12}H_{18}N_2O_3S$: 270.35
N-(Butylcarbamoyl)-4-methylbenzenesulfonamide
[*64-77-7*]

Tolbutamide, when dried, contains not less than 99.0% of $C_{12}H_{18}N_2O_3S$.

Description Tolbutamide occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** Boil 0.2 g of Tolbutamide with 8 mL of diluted sulfuric acid (1 in 3) under a reflux condenser for 30 minutes. Cool the solution in ice water, collect the precipitated crystals, recrystallize from water, and dry at 105°C for 3 hours: the crystals melt ⟨*2.60*⟩ between 135°C and 139°C.

(2) Render the filtrate obtained in (1) alkaline with about 20 mL of a solution of sodium hydroxide (1 in 5), and heat: an ammonia-like odor is perceptible.

Melting point ⟨*2.60*⟩ 126 – 132°C

Purity **(1)** Acidity—Warm 3.0 g of Tolbutamide with 150 mL of water at 70°C for 5 minutes, allow to stand for 1 hour in ice water, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Chloride ⟨*1.03*⟩—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 ⟨*1.14*⟩—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 ⟨*1.07*⟩—Proceed with 2.0 g of Tolbutamide 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).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tolbutamide, previously dried, and dissolve in 30 mL of neutralized ethanol. Add 20 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.04 mg of $C_{12}H_{18}N_2O_3S$

Containers and storage Containers—Well-closed containers.

Tolbutamide Tablets

トルブタミド錠

Tolbutamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$: 270.35).

Method of preparation Prepare as directed under Tablets, with Tolbutamide.

Identification Shake a quantity of powdered Tolbutamide Tablets, equivalent to 0.5 g of Tolbutamide according to the labeled amount, with 50 mL of chloroform, filter, and evaporate the filtrate to dryness. Proceed with the residue as directed in the Identification under Tolbutamide.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of phosphate buffer solution, pH 7.4 as the dissolution medium, the dissolution rate in 30 minutes of Tolbutamide Tablets is not less than 80%.

Start the test with 1 tablet of Tolbutamide Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of tolbutamide ($C_{12}H_{18}N_2O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tolbutamide RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add the dissolution medium 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 the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the control, and determine the absorbances, A_T and A_S, at 226 nm.

Dissolution rate (%) with respect to the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Tolbutamide RS
C: Labeled amount (mg) of tolbutamide ($C_{12}H_{18}N_2O_3S$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Tol-

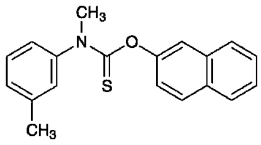
butamide Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of tolbutamide ($C_{12}H_{18}N_2O_3S$), dissolve in 50 mL of neutralized ethanol, add 25 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.04 mg of $C_{12}H_{18}N_2O_3S$

Containers and storage Containers—Well-closed containers.

Tolnaftate

トルナフタート



$C_{19}H_{17}NOS$: 307.41
O-Naphthalen-2-yl *N*-methyl-*N*-(3-methylphenyl)thiocarbamate
[*2398-96-1*]

Tolnaftate, when dried, contains not less than 98.0% of $C_{19}H_{17}NOS$.

Description Tolnaftate occurs as a white powder. It is odorless.

It is freely soluble in chloroform, sparingly soluble in diethyl ether, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** To 0.2 g of Tolnaftate add 20 mL of potassium hydroxide-ethanol TS and 5 mL of water, and heat under a reflux condenser for 3 hours. After cooling, to 10 mL of this solution add 2 mL of acetic acid (100), and shake with 1 mL of lead (II) acetate TS: a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Tolnaftate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolnaftate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolnaftate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolnaftate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 111 – 114°C (after drying).

Purity **(1)** Heavy metals <*1.07*>—Carbonize 1.0 g of Tolnaftate by gentle heating. After cooling, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid and 0.5 mL of perchloric acid, and heat gradually until white fumes are evolved. Repeat this procedure twice, and heat until white fumes are no longer evolved. Incinerate the residue by igniting between 500°C and 600°C for 1 hour. Proceed according to Method 2, and perform the test with 50 mL of the test solution so obtained. Prepare the control solution as follows: to 11 mL of nitric acid add 1 mL of sulfuric acid, 1 mL of perchloric acid and 2 mL of hydrochloric acid, proceed in the same manner as the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.50 g of Tolnaftate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes, and examine under ultraviolet light (wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 65°C, 3 hours).

Residue on ignition <*2.44*> Weigh accurately about 2.0 g of Tolnaftate, and carbonize by gradual heating. Moisten the substance with 1 mL of sulfuric acid, heat gradually until white fumes are no longer evolved, and ignite between 450°C and 550°C for about 2 hours to constant mass: the residue is not more than 0.1%.

Assay Weigh accurately about 50 mg of Tolnaftate and Tolnaftate RS, previously dried, dissolve each in 200 mL of methanol by warming in a water bath, cool, and add methanol to make exactly 250 mL. Pipet 5 mL each of the solutions, to each add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{19}H_{17}NOS = M_S \times A_T/A_S$$

M_S: Amount (mg) of Tolnaftate RS

Containers and storage Containers—Tight containers.

Tolnaftate Solution

トルナフタート液

Tolnaftate Solution contains not less than 90.0% and not more than 110.0% of the labeled amount of tolnaftate ($C_{19}H_{17}NOS$: 307.41).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Tolnaftate.

Identification **(1)** Spot 1 drop of Tolnaftate Solution on filter paper. Spray hydrogen hexachloroplatinate (IV)-potas-

sium iodide TS on the paper: a light yellow color develops in the spot.

(2) To a volume of Tolnaftate Solution, equivalent to 0.02 g of Tolnaftate according to the labeled amount, add chloroform to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.02 g of Tolnaftate RS in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R*f value.

Assay Pipet a volume of Tolnaftate Solution, equivalent to about 20 mg of tolnaftate ($C_{19}H_{17}NOS$), add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of Tolnaftate RS, previously dried in vacuum at a pressure not exceeding 0.67 kPa at 65°C for 3 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tolnaftate to that of the internal standard, respectively.

$$\text{Amount (mg) of tolnaftate } (C_{19}H_{17}NOS) = M_S \times Q_T/Q_S \times 1/20$$

M_S: Amount (mg) of Tolnaftate RS

Internal standard solution—A solution of diphenyl phthalate in chloroform (3 in 200).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 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: A mixture of methanol and water (7:3).

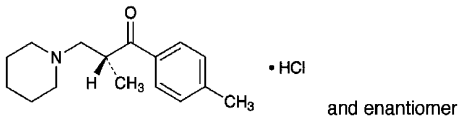
Flow rate: Adjust the flow rate so that the retention time of tolnaftate is about 14 minutes.

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 the internal standard and tolnaftate in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Tolperisone Hydrochloride

トルペリゾン塩酸塩



and enantiomer

$C_{16}H_{23}NO.HCl$: 281.82

(2*RS*)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one monohydrochloride

[*3644-61-9*]

Tolperisone Hydrochloride, when dried, contains not less than 98.5% of $C_{16}H_{23}NO.HCl$.

Description Tolperisone Hydrochloride occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in diethyl ether.

The pH of a solution of Tolperisone Hydrochloride (1 in 20) is between 4.5 and 5.5.

It is hygroscopic.

Melting point: 167 – 174°C

Identification **(1)** Dissolve 0.2 g of Tolperisone Hydrochloride in 2 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS, and heat: a red color develops.

(2) To 5 mL of a solution of Tolperisone Hydrochloride (1 in 20) add 2 to 3 drops of iodine TS: a red-brown precipitate is produced.

(3) Dissolve 0.5 g of Tolperisone Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. Acidify 5 mL of the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E^{1\%}_{1\,cm}$ (257 nm): 555 – 585 (after drying, 5 mg, ethanol (95), 500 mL).

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

(2) Sulfate <1.14>—Perform the test using 4.0 g of Tolperisone Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Tolperisone 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) Piperidine hydrochloride—Dissolve 0.20 g of Tolperisone Hydrochloride in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of piperidine hydrochloride in water to make exactly 1000 mL, and use this solution as the standard solution. Transfer 5.0 mL each of the sample solution and standard solution to different separators, add 0.1 mL each of a solution of copper (II) sulfate pentahydrate (1 in 20), then add 0.1 mL each of ammonia solution (28) and exactly 10 mL each of a mixture of isooctane and carbon disulfide (3:1),

and shake vigorously for 30 minutes. Immediately after allowing to stand, separate the isooctane-carbon disulfide mixture layer, and dehydrate with anhydrous sodium sulfate. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance of the sample solution at 438 nm is not more than that of the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

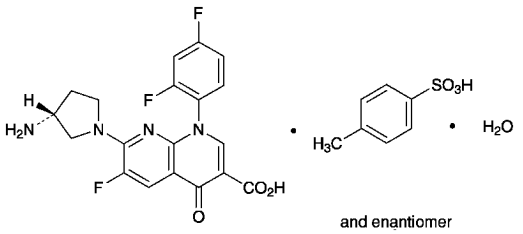
Assay Weigh accurately about 0.5 g of Tolperisone Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.18 mg $C_{16}H_{23}NO.HCl$

Containers and storage Containers—Well-closed containers.

Tosufloxacin Tosilate Hydrate

トスフロキサシントシル酸塩水和物



$C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$: 594.56

7-[(3*RS*)-3-Aminopyrrolidin-1-yl]-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid mono-4-toluenesulfonate monohydrate

[*115964-29-9*, anhydride]

Tosufloxacin Tosilate Hydrate contains not less than 98.5% and not more than 101.0% of tosufloxacin tosilate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S$: 576.54), calculated on the anhydrous basis.

Description Tosufloxacin Tosilate Hydrate occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in methanol, and practically insoluble in water and in ethanol (99.5).

A solution of Tosufloxacin Tosilate Hydrate in methanol (1 in 100) shows no optical rotation.

Melting point: about 254°C (with decomposition).

Identification **(1)** Tosufloxacin Tosilate Hydrate shows a light bluish-white fluorescence under ultraviolet light (main wavelength 254 nm).

(2) Proceed 10 mg of Tosufloxacin Tosilate Hydrate as directed under Oxygen Flask Combustion Method <*1.06*>, 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 <*1.09*> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Tosufloxacin Tosilate Hydrate in a mixture of methanol and sodium hydroxide TS (49:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tosufloxacin Tosilate Hydrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tosufloxacin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tosufloxacin Tosilate Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Tosufloxacin Tosilate Hydrate in 40 mL of *N*,*N*-dimethylformamide, and add 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide 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, 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide to make 50 mL (not more than 0.007%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tosufloxacin Tosilate 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).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Tosufloxacin Tosilate Hydrate according to Method 4, and perform the test under the condition of the ignition temperature being between 750 and 850°C, and add 10 mL of diluted hydrochloric acid to residue (not more than 2 ppm).

(4) Related substances—Dissolve 10 mg of Tosufloxacin Tosilate Hydrate in 12 mL of mobile phase B, add water to make 25 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase A to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the area of each peak other than tosylic acid and tosufloxacin obtained from the sample solution is not larger than 3/4 times the peak area of tosufloxacin from the standard solution, and the total area of the peaks other than tosylic acid and tosufloxacin from the sample solution is not larger than 2.5 times the peak area of tosufloxacin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 3.0 mm in inside 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°C.

Mobile phase A: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and

add water to make 1000 mL. To 10 mL of this solution add 143 mL of water, 40 mL of acetonitrile and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Mobile phase B: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 100 mL of acetonitrile, 83 mL of water and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 1	100	0
1 – 16	100 → 0	0 → 100
16 – 35	0	100

Flow rate: 0.5 mL per minute.

Time span of measurement: About 5 times as long as the retention time of tosufloxacin.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add mobile phase A to make exactly 20 mL. Confirm that the peak area of tosufloxacin obtained from 20 μL of this solution is equivalent to 18 to 32% of the peak area of tosufloxacin 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 symmetry factor of the peak of tosufloxacin are not less than 10,000 and not more than 1.5, respectively.

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

(5) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ 2.5 – 3.5% (30 mg, coulometric titration).

Assay Weigh accurately about 30 mg each of Tosufloxacin Tosilate Hydrate and Tosufloxacin Tosilate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve each in methanol to make exactly 100 mL. Pipet 20 mL each of these solutions, to each add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tosufloxacin to that of the internal standard.

$$\text{Amount (mg) of tosufloxacin tosilate } (C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To a mixture of 0.02 mol/L phosphate buffer solution, pH 3.5, and a solution of dibutylamine in methanol (1 in 2500) (3:1) add diluted phosphoric acid (1 in 10) to adjust the pH to 3.5.

Flow rate: Adjust the flow rate so that the retention time of tosufloxacin is about 20 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 tosufloxacin 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tosufloxacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Tosufloxacin Tosilate Tablets

トスフロキサシントシル酸塩錠

Tosufloxacin Tosilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tosuflozacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$: 594.56).

Method of preparation Prepare as directed under Tablets, with Tosufloxacin Tosilate Hydrate.

Identification To a quantity of powdered Tosufloxacin Tosilate Tablets, equivalent to 75 mg of Tosufloxacin Tosilate Hydrate according to the labeled amount, add 200 mL of a mixture of methanol and sodium hydroxide TS (49:1), shake well, and centrifuge. To 2 mL of the supernatant liquid add 100 mL of a mixture of methanol and sodium hydroxide TS (49:1). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 260 nm and 264 nm, between 341 nm and 345 nm, and between 356 nm and 360 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tosufloxacin Tosilate Tablets add $V/10$ mL of water and shake until the tablet is disintegrated. Add methanol to make exactly V mL so that each mL contains about 1.5 mg of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$). Shake this solution for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$)
$= M_S \times Q_T/Q_S \times V/20 \times 1.031$

M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Tosufloxacin Tosilate Tablets is not less than 65%.

Start the test with 1 tablet of Tosufloxacin Tosilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting 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 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly V' mL so that each mL contains about 17 μg of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Tosufloxacin Tosilate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve in *N*,*N*-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the blank, and determine the absorbances, A_T and A_S, at 346 nm.

Dissolution rate (%) with respect to the labeled amount of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72 \times 1.031$

M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis
C: Labeled amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Tosufloxacin Tosilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$), add 10 mL of water and methanol to make exactly 100 mL, shake for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Tosufloxacin Tosilate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Tosufloxacin Tosilate Hydrate), add 2 mL of water, and dissolve in methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tosufloxacin to that of the internal standard.

Amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$)
$= M_S \times Q_T/Q_S \times 5 \times 1.031$

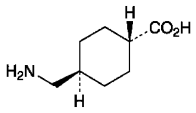
M_S: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Tosufloxacin Tosilate Hydrate.
System suitability—
Proceed as directed in the system suitability in the Assay under Tosufloxacin Tosilate Hydrate.

Containers and storage Containers—Well-closed containers.

Tranexamic Acid

トラネキサム酸



$C_8H_{15}NO_2$: 157.21
trans-4-(Aminomethyl)cyclohexanecarboxylic acid
[*1197-18-8*]

Tranexamic Acid, when dried, contains not less than 98.0% and not more than 101.0% of $C_8H_{15}NO_2$.

Description Tranexamic Acid occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Tranexamic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Tranexamic Acid RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.0 g of Tranexamic Acid in 20 mL of water is between 7.0 and 8.0.

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

(2) Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of Tranexamic Acid. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals—Dissolve 2.0 g of Tranexamic Acid in water to make 20 mL, and use this solution as the sample stock solution. To 12 mL of the sample stock solution add 2 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, mix, add 1.2 mL of thioacetamide TS , mix immediately, and use this solution as the sample solution. Sepa-

rately, proceed in the same manner as above with a mixture of 1 mL of Standard Lead Solution, 2 mL of the sample stock solution and 9 mL of water, and use the solution so obtained as the standard solution. Separately, proceed in the same manner with a mixture of 10 mL of water and 2 mL of the sample stock solution, and use the solution so obtained as the control solution. Conform that the color of the standard solution is slightly darker than that of the control solution. Compare the sample solution and the standard solution 2 minutes after they are prepared: the color of the sample solution is not more intense than that of the standard solution (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution by dissolving 1.0 g of Tranexamic Acid in 10 mL of water, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Tranexamic Acid in 20 mL of water, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area multiplied by relative response factor 1.2 of the peak, having the relative retention time of about 1.5 with respect to tranexamic acid obtained from sample solution, is not larger than 2/5 times the peak area of tranexamic acid from the standard solution, and the area of the peak, having the relative retention time of about 2.1, is not larger than 1/5 times the peak area of tranexamic acid from the standard solution. The area of each peak other than tranexamic acid and other than the peaks mentioned above is not larger than 1/5 times the peak area of tranexamic acid from the standard solution. For this comparison, use the area of the peaks, having the relative retention time of about 1.1 and about 1.3, after multiplying by their relative response factors 0.005 and 0.006, respectively. The total area of the peaks other than tranexamic acid is not larger than the peak area of tranexamic acid 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.

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

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 25 mL. Confirm that the peak area of tranexamic acid obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 7%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Tranexamic Acid and Tranexamic Acid RS, previously dried, dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid.

$$\text{Amount (mg) of } C_8H_{15}NO_2 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside 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 11.0 g of anhydrous sodium dihydrogen phosphate in 500 mL of water, and add 5 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid or diluted phosphoric acid (1 in 10), add water to make 600 mL, and add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 20 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 0.6%.

Containers and storage Containers—Well-closed containers.

Tranexamic Acid Capsules

トラネキサム酸カプセル

Tranexamic Acid Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Capsules, with Tranexamic Acid.

Identification Take an amount of powdered contents of Tranexamic Acid Capsules, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement

of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Tranexamic Acid Capsules is not less than 80%.

Start the test with 1 tablet of Tranexamic Acid Capsules, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.28 mg of tranexamic acid ($C_8H_{15}NO_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of tranexamic acid, A_T and A_S.

Dissolution rate (%) with respect to the labeled amount of tranexamic acid ($C_8H_{15}NO_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 900$

M_S: Amount (mg) of Tranexamic Acid RS
C: Labeled amount (mg) of tranexamic acid ($C_8H_{15}NO_2$) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 11.0 g of anhydrous sodium dihydrogen phosphate in 500 mL of water, and add 10 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid, add water to make 600 mL, and add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid 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 and the symmetry factor of the peak of tranexamic acid are not less than 4000 and not more than 2.0, respectively.

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

Assay Weigh accurately the mass of the contents of not less than 20 Tranexamic Acid Capsules, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tranexamic acid ($C_8H_{15}NO_2$), add 30 mL of water, shake well, and add water to make exactly 50 mL. Centrifuge, filter the supernatant liquid through a membrane filter with pore size of not more than 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid.

Amount (mg) of tranexamic acid ($C_8H_{15}NO_2$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Tranexamic Acid Injection

トラネキサム酸注射液

Tranexamic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Injections, with Tranexamic Acid.

Description Tranexamic Acid Injection is a clear and colorless liquid.

Identification To a volume of Tranexamic Acid Injection, equivalent to 50 mg of Tranexamic Acid according to the labeled amount, add water to make 5 mL, add 1 mL of ninhydrin TS, and heat: a dark purple color develops.

pH ⟨*2.54*⟩ 7.0 – 8.0

Bacterial endotoxins ⟨*4.01*⟩ Not more than 0.12 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take accurately a volume of Tranexamic Acid Injection, equivalent to about 0.1 g of tranexamic acid ($C_8H_{15}NO_2$), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105 °C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid.

$$\text{Amount (mg) of tranexamic acid } (C_8H_{15}NO_2) = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Tranexamic Acid Tablets

トラネキサム酸錠

Tranexamic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Tablets, with Tranexamic Acid.

Identification To an amount of powdered Tranexamic Acid Tablets, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tranexamic Acid Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 5 g of tranexamic acid ($C_8H_{15}NO_2$), add 150 mL of water, disintegrate the tablets completely with the aid of ultrasonic waves, and add water to make exactly 200 mL. Centrifuge, pipet 4 mL of the supernatant liquid, and add water to make exactly 50 mL. Filter through a membrane filter with pore size of not more than 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid.

$$\text{Amount (mg) of tranexamic acid } (C_8H_{15}NO_2) = M_S \times A_T/A_S \times 100$$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

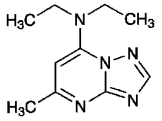
System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trapidil

トラピジル



$C_{10}H_{15}N_5$: 205.26

7-Diethylamino-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine [*15421-84-8*]

Trapidil, when dried, contains not less than 98.5% of $C_{10}H_{15}N_5$.

Description Trapidil occurs as a white to pale yellowish

white, crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), in acetic anhydride and in acetic acid (100), and sparingly soluble in diethyl ether.

The pH of a solution of Trapidil (1 in 100) is between 6.5 and 7.5.

Identification **(1)** To 5 mL of a solution of Trapidil (1 in 50) add 3 drops of Dragendorff's TS: an orange color develops.

(2) Determine the absorption spectrum of a solution of Trapidil (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (307 nm): 860 – 892 (after drying, 20 mg, water, 2500 mL).

Melting point <*2.60*> 101 – 105°C

Purity **(1)** Clarity and color of solution—Dissolve 2.5 g of Trapidil in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Trapidil. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Ammonium—Place 0.05 g of Trapidil in a glass-stoppered conical flask, thoroughly moisten with 10 drops of sodium hydroxide TS, and stopper the flask. Allow it to stand at 37°C for 15 minutes: the gas evolved does not change moistened red litmus paper to blue.

(4) Heavy metals <*1.07*>—Dissolve 1.0 g of Trapidil in 40 mL of water, and add 1.5 mL of dilute hydrochloric acid, 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 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Trapidil according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of Trapidil in 4 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 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 <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (85:13:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 60 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

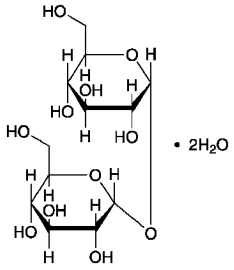
Assay Weigh accurately about 0.2 g of Trapidil, previously dried, dissolve in 20 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.53 mg of $C_{10}H_{15}N_5$

Containers and storage Containers—Tight containers.

Trehalose Hydrate

トレハロース水和物



$C_{12}H_{22}O_{11}.2H_2O$: 378.33
α-D-Glucopyranosyl α-D-glucopyranoside dihydrate
[*6138-23-4*]

Trehalose Hydrate contains not less than 98.0% and not more than 101.0% of trehalose ($C_{12}H_{22}O_{11}$: 342.30), calculated on the anhydrous basis.

Description Trehalose Hydrate occurs as white crystals or a white crystalline powder.

It is freely soluble in water, and slightly soluble in methanol and in ethanol (99.5).

Identification **(1)** To 1 mL of a solution of Trehalose Hydrate (2 in 5) add 5 – 6 drops of a solution of 1-naphthol in ethanol (95) (1 in 20), shake thoroughly, and add gently 2 mL of sulfuric acid: a purple color appears at the zone of contact.

(2) Mix 2 mL of a solution of Trehalose Hydrate (1 in 25) with 1 mL of dilute hydrochloric acid, and allow standing for 20 minutes at room temperature. Then add 4 mL of sodium hydroxide TS and 2 mL of a solution of glycine (1 in 25), and heat in a water bath for 10 minutes: no brown color appears.

(3) Determine the infrared absorption spectrum of Trehalose Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Trehalose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +197 – +201° (10 g, calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <*2.54*> The pH of a solution of 1 g of Trehalose Hydrate in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Chloride <*1.03*>—Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Sulfate <*1.14*>—Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of

0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of Trehalose Hydrate 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) Related substances—Dissolve 0.5 g of Trehalose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks which are eluted before the peak of trehalose and the total area of the peaks which are eluted after the peak of trehalose obtained from the sample solution are both not larger than 1/2 times the peak area of trehalose from the standard solution.

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time of trehalose.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL. Confirm that the peak area of trehalose obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

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

System repeatability: To exactly 5 mL of the standard solution add water to make exactly 10 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of trehalose is not more than 1.0%.

(5) Dextrin, soluble starch, and sulfite—Dissolve 1.0 g of Trehalose Hydrate in 10 mL of water and add 1 drop of iodine TS: a yellow color appears, which is changed to blue on addition of 1 drop of starch TS.

(6) Nitrogen—Perform the test with accurately weighed Trehalose Hydrate of about 5 g as directed under Nitrogen Determination ⟨*1.08*⟩, using 30 mL of sulfuric acid for the degradation and adding 45 mL of sodium hydroxide solution (2 in 5): the amount of nitrogen (N: 14.01) is not more than 0.005%.

Water ⟨*2.48*⟩ not less than 9.0% and not more than 11.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (2 g).

Assay Weigh accurately about 0.2 g each of Trehalose Hydrate and Trehalose RS (separately determine the water ⟨*2.48*⟩ in the same manner as Trehalose Hydrate), dissolve each in 6 mL of water, add exactly 2 mL each of the internal standard solution, add water to make them 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of trehalose to that of the internal standard.

Amount (mg) of trehalose ($C_{12}H_{22}O_{11}$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Trehalose RS, calculated on the anhydrous basis

Internal standard solution—A solution of glycerin (1 in 10).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography consist of styrene-divinylbenzene copolymer carrying sulfonic acid groups (6 μm in particle diameter).

Column temperature: A constant temperature of about 80°C.

Mobile phase: Water.

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

System suitability—

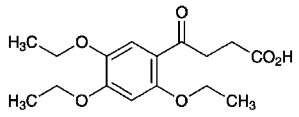
System performance: Dissolve 0.1 g each of maltotriose and glucose in 10 mL of the standard solution, add 1 mL of the internal standard solution, and add water to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, maltotriose, trehalose, glucose and the internal standard are eluted in this order, and the resolution between the peaks of maltotriose and trehalose is not less than 1.5, the resolution between the peaks of trehalose and glucose is not less than 4, and the resolution between the peaks of glucose and the internal standard is not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trehalose to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trepibutone

トレピブトン



$C_{16}H_{22}O_6$: 310.34

4-Oxo-4-(2,4,5-triethoxyphenyl)butanoic acid

[*41826-92-0*]

Trepibutone, when dried, contains not less than 98.5% of $C_{16}H_{22}O_6$.

Description Trepibutone occurs as white to yellowish white crystals or crystalline powder. It is odorless, and is tasteless or has a slight, characteristic aftertaste.

It is soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Trepibutone in diluted dilute sodium hydroxide TS (1 in 10) (1 in 100,000) as directed under Ultraviolet-

visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Trepibutone in deuterated chloroform for the nuclear magnetic resonance spectroscopy (1 in 10), using tetramethylsilane for the nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>: it exhibits a sharp multiple signal A at around δ 1.5 ppm, a triplet signal B at around δ 2.7 ppm, a triplet signal C at around δ 3.3 ppm, a multiple signal D at around δ 4.2 ppm, a sharp single signal E at around δ 6.4 ppm, a sharp single signal F at around δ 7.4 ppm, and a single signal G at around δ 10.5 ppm. The ratio of integrated intensity of each signal, A:B:C:D:E:F:G, is about 9:2:2:6:1:1:1.

Melting point <2.60> 146 – 150°C

Purity **(1)** Chloride <1.03>—Dissolve 0.5 g of Trepibutone 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 0.30 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 0.021%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Trepibutone 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 0.10 g of Trepibutone in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 100 mL. To exactly 10 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 Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, acetone, water and formic acid (100:30:3:3) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

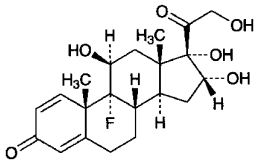
Assay Weigh accurately about 0.5 g of Trepibutone, previously dried, dissolve in 50 mL of ethanol (95), add 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.03 mg of $C_{16}H_{22}O_6$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Triamcinolone

トリアムシノロン



$C_{21}H_{27}FO_6$: 394.43
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione
[*124-94-7*]

Triamcinolone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{27}FO_6$.

Description Triamcinolone occurs as a white, crystalline powder. It is odorless.

It is freely soluble in *N*,*N*-dimethylformamide, slightly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water, in 2-propanol and in diethyl ether.

Melting point: about 264°C (with decomposition).

Identification **(1)** Dissolve 1 mg of Triamcinolone in 6 mL of ethanol (95), add 5 mL of 2,6-di-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath for 30 minutes under a reflux condenser: a red-purple color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone as directed under Oxygen Flask Combustion Method <1.06>, 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 <1.09> for fluoride.

(4) Determine the infrared absorption spectrum of Triamcinolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone and Triamcinolone RS in 7 mL of a mixture of 2-propanol and water (2:1), respectively, by warming. Allow the solutions to cool in ice to effect crystals, filter, then wash the formed crystals with two 10-mL portions of water, and repeat the test on the dried crystals.

Optical rotation <2.49> $[\alpha]_D^{20}$: +65 – +71° (after drying, 0.1 g, *N*,*N*-dimethylformamide, 10 mL, 100 mm).

Purity Heavy metals <1.07>—Proceed with 0.5 g of Triamcinolone 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).

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (0.5 g,

platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone and Triamcinolone RS, previously dried and accurately weighed, in a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of triamcinolone to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{27}FO_6 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Triamcinolone RS

Internal standard solution—Dissolve 15 mg of methyl parahydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make 100 mL.

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

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

Flow rate: Adjust the flow rate so that the retention time of triamcinolone 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, triamcinolone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

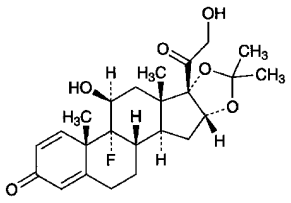
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Triamcinolone Acetonide

トリアムシノロンアセトニド



$C_{24}H_{31}FO_6$: 434.50

9-Fluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione

[*76-25-5*]

Triamcinolone Acetonide, when dried, contains not less than 97.0% and not more than 103.0% of $C_{24}H_{31}FO_6$.

Description Triamcinolone Acetonide occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (99.5), in acetone, and in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Melting point: about 290°C (with decomposition).

Identification **(1)** Dissolve 2 mg of Triamcinolone Acetonide in 40 mL of ethanol (95), add 5 mL of 2,6-di-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath under a reflux condenser for 20 minutes: a green color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone Acetonide, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone Acetonide as directed under Oxygen Flask Combustion Method <*1.06*>, 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 <*1.09*> for fluoride.

(4) Determine the absorption spectrum of a solution of Triamcinolone Acetonide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Triamcinolone Acetonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Triamcinolone Acetonide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone Acetonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone Acetonide and Triamcinolone Acetonide RS in 20 mL of ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the dried residue.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +100 – +107° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of

Triamcinolone Acetonide 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 40 mg of Triamcinolone Acetonide in 4 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample 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 Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample 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 and methanol (93:7) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g, platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone Acetonide and Triamcinolone Acetonide RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of triamcinolone acetonide to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{24}H_{31}FO_6 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Triamcinolone Acetonide RS

Internal standard solution—A solution of prednisolone in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

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

Flow rate: Adjust the flow rate so that the retention time of triamcinolone acetonide is about 13 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 triamcinolone acetonide are eluted in this order with the resolution between these peaks being not less than 6.

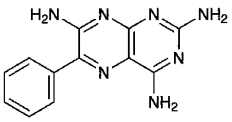
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Triamterene

トリアムテレン



$C_{12}H_{11}N_7$: 253.26

6-Phenylpteridine-2,4,7-triamine

[*396-01-0*]

Triamterene, when dried, contains not less than 98.5% of $C_{12}H_{11}N_7$.

Description Triamterene occurs as a yellow, crystalline powder. It is odorless, and tasteless.

It is sparingly soluble in dimethylsulfoxide, very slightly soluble in acetic acid (100), and practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in nitric acid and in sulfuric acid, but does not dissolve in dilute nitric acid, in dilute sulfuric acid and in dilute hydrochloric acid.

Identification **(1)** To 0.01 g of Triamterene add 10 mL of water, heat, and filter after cooling: the filtrate shows a purple fluorescence. To 2 mL of the filtrate add 0.5 mL of hydrochloric acid: the fluorescence disappears.

(2) The filtrate obtained in (1) responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) Dissolve 0.01 g of Triamterene in 100 mL of acetic acid (100), and to 10 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Triamterene 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 <*1.11*>—Prepare the test solution with 1.0 g of Triamterene according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Triamterene in 20 mL of dimethylsulfoxide. To 2 mL of this solution add methanol to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (9:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.10% (1 g).

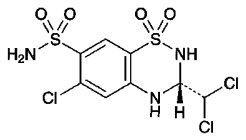
Assay Weigh accurately about 0.15 g of Triamterene, previously dried, and dissolve in 100 mL of acetic acid (100) by warming. Titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.66 mg of $C_{12}H_{11}N_7$

Containers and storage Containers—Well-closed containers.

Trichlormethiazide

トリクロルメチアジド



and enantiomer

$C_8H_8Cl_3N_3O_4S_2$: 380.66
(3*RS*)-6-Chloro-3-dichloromethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
[*133-67-5*]

Trichlormethiazide, when dried, contains not less than 97.5% and not more than 102.0% of $C_8H_8Cl_3N_3O_4S_2$.

Description Trichlormethiazide occurs as a white powder.

It is freely soluble in *N*,*N*-dimethylformamide and in acetone, slightly soluble in acetonitrile and in ethanol (95), and practically insoluble in water.

A solution of Trichlormethiazide in acetone (1 in 50) shows no optical rotation.

Melting point: about 270°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Trichlormethiazide in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Trichlormethiazide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trichlormethiazide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Trichlormethiazide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Trichlormethiazide as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Trichlormethiazide 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 nitiric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate ⟨*1.14*⟩—Dissolve 1.0 g of Trichlormethiazide 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 ⟨*1.07*⟩—Proceed with 1.0 g of Trichlormethiazide 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 ⟨*1.11*⟩—Prepare the test solution with 0.6 g of Trichlormethiazide according to Method 5, using 20 mL of *N*,*N*-dimethylformamide, and perform the test (not more than 3.3 ppm).

(5) Related substances—Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile, and use the solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of related substances by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 2.0%, and the total amount of the related substances is not more than 2.5%.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 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 A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 20	100 → 0	0 → 100

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the

peak area of trichlormethiazide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that from 10 μL of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath at 60°C for 30 minutes. When the procedure is run with 10 μL of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg of Trichlormethiazide and Trichlormethiazide RS, previously dried, and dissolve separately in exactly 20 mL of the internal standard solution. To 1 mL of these solutions add acetonitrile to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of trichlormethiazide to that of the internal standard.

$$\text{Amount (mg) of } C_8H_8Cl_3N_3O_4S_2 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Trichlormethiazide RS

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 800).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 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: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide 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 trichlormethiazide 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Trichlormethiazide Tablets

トリクロルメチアジド錠

Trichlormethiazide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$: 380.66).

Method of preparation Prepare as directed under Tablets, with Trichlormethiazide.

Identification To an amount of pulverized Trichlormethiazide Tablets, equivalent to 4 mg of Trichlormethiazide according to the labeled amount, add 10 mL of acetone, shake vigorously for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 4 mg of Trichlormethiazide RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample 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 and methanol (10:4:1) 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 sample solution and the standard solution show the same *R*f value.

Purity Related substances—Pulverize a suitable amount of Trichlormethiazide Tablets in an agate mortar. Take an amount of the powder, equivalent to 10 mg of Trichlormethiazide according to the labeled amount, add 20 mL of acetonitrile, shake vigorously for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of each related substance by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfoneamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 4.0%, and the total amount of the peaks other than trichlormethiazide is not more than 5.0%.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 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 A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 20	100 → 0	0 → 100

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μL of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath of 60°C for 30 minutes. When the procedure is run with 10 μL of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trichlormethiazide Tablets add 5 mL of diluted phosphoric acid (1 in 50) to disintegrate. Add exactly an amount of the internal standard solution, equivalent to 10 mL per 2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$), add acetonitrile to make 25 mL, shake vigorously for 15 minutes, and centrifuge. To an amount of the supernatant liquid add the mobile phase to make a solution so that it contains about 40 μg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), 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 ⟨*2.01*⟩ according to the conditions described in the Assay, and calculate the ratios, Q_T and Q_S, of the peak area of trichlormethiazide to that of the internal standard.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)
$= M_S \times Q_T/Q_S \times C \times 1/20$

M_S: Amount (mg) of Trichlormethiazide RS
C: Labeled amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) in 1 tablet

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Trichlormethiazide Tablets is not less than 75%.

Start the test with 1 tablet of Trichlormethiazide Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add diluted phosphoric acid (1 in 50) to make exactly V' mL so that each mL contains about 1.1 μg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 2 mL of this solution, add diluted phosphoric acid (1 in 50) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{Ta} and A_{Sa}, of trichlormethiazide obtained with the sample solution and standard solution, and the area, A_{Tb}, of the peak, having the relative retention time of about 0.3 to trichlormethiazide, obtained with the sample solution.

Dissolution rate (%) with respect to the labeled amount of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)
$= M_S \times (A_{Ta} + 0.95A_{Tb})/A_{Sa} \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of Trichlormethiazide RS
C: Labeled amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL. To 5 mL of this solution add 5 mL of water, and heat at 60°C in a water bath for 30 minutes. After cooling, when the procedure is run with 10 μL of this solution under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Trichlormethiazide Tablets, and pulverize the tablets in

an agate mortar. Weigh accurately an amount of the powder, equivalent to about 2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$), add 5 mL of diluted phosphoric acid (1 in 50) and exactly 10 mL of the internal standard solution, add 10 mL of acetonitrile, shake vigorously for 15 minutes, and centrifuge. To 2 mL of the supernatant liquid add 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of trichlormethiazide to that of the internal standard.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)
$= M_S \times Q_T/Q_S \times 1/20$

M_S: Amount (mg) of Trichlormethiazide RS

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 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: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutes.

System suitability—

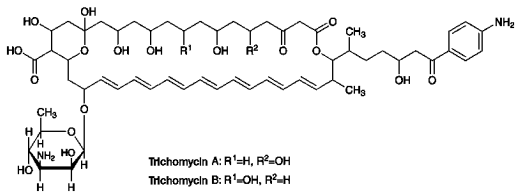
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trichomycin

トリコマイシン



Trichomycin A
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,9,11,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid
[*12698-99-6*]
Trichomycin B
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,7,9,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid
[*12699-00-2*]
[*1394-02-1*, Trichomycin]

Trichomycin is a mixture of polyene macrolide substances having antifungal and antiprotozoal activities produced by the growth of *Streptomyces hachijoensis*.

It contains not less than 7000 Units per mg, calculated on the dried basis. The potency of Trichomycin is expressed as unit based on the amount of trichomycin. One unit of Trichomycin is equivalent to 0.05 μg of trichomycin.

Description Trichomycin occurs as a yellow to yellow-brown powder.

It is practically insoluble in water, in ethanol (99.5) and in tetrahydrofuran.

It dissolves in dilute sodium hydroxide TS.

It is hygroscopic.

Identification **(1)** To 2 mg of Trichomycin add 2 mL of sulfuric acid: a blue color appears, and the color is changed to a blue-purple after allowing to stand.

(2) Dissolve 1 mg of Trichomycin in 50 mL of a solution of sodium hydroxide (1 in 200). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 359 nm and 365 nm, between 378 nm and 384 nm, and between 400 nm and 406 nm.

Content ratio of the active principle Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve about 10 mg of Trichomycin in 50 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amount of trichomycin A and trichomycin B by the area percentage

method: the amount of trichomycin A is between 20% and 40%, and that of trichomycin B is between 15% and 25%. The relative retention time of trichomycin B with respect to trichomycin A is about 1.2.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in a mixture of 600 mL of water and 400 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of trichomycin A is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of trichomycin A.

System suitability—

Test for required detectability: Measure 5 mL of the sample solution, add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 30 mL. Confirm that the peak area of trichomycin A obtained from 5 μL of this solution is equivalent to 12 to 22% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, trichomycin A and trichomycin B 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 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichomycin A is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately an amount of Trichomycin and Trichomycin RS, equivalent to about 150,000 units, dissolve them separately in a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of trichomycin.

$$\text{Amount (unit) of trichomycin} = M_S \times A_T/A_S$$

M_S: Amount (unit) of Trichomycin RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 15 g of ammonium acetate in 120 mL of water, and add 1000 mL of acetonitrile for liquid chromatography and 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of trichomycin is about 6 minutes.

System suitability—

System performance: Dissolve 5 mg of Trichomycin and 1 mg of berberine chloride in 100 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1). When the procedure is run with 20 μL of this solution under the above operating conditions, berberine and trichomycin 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichomycin is not more than 2.0%.

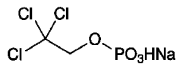
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Triclofos Sodium

Monosodium Trichloroethyl Phosphate

トリクロホスナトリウム



$C_2H_3Cl_3NaO_4P$: 251.37

Monosodium 2,2,2-trichloroethyl monohydrogenphosphate [*7246-20-0*]

Triclofos Sodium, when dried, contains not less than 97.0% and not more than 102.0% of $C_2H_3Cl_3NaO_4P$, and not less than 41.0% and not more than 43.2% of chlorine (Cl: 35.45).

Description Triclofos Sodium is a white, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Triclofos Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 0.5 g of Triclofos Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite further over a flame. Dissolve the residue in 5 mL of water, and filter it necessary: the filtrate responds to Qualitative Tests ⟨*1.09*⟩ for sodium salt.

(3) To 0.1 g of Triclofos Sodium add 1 g of anhydrous sodium carbonate, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

The remainder of the filtrate responds to the Qualitative Tests <*1.09*> (1) for chloride and to the Qualitative Tests <*1.09*> for phosphate.

pH <*2.54*> Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the pH of this solution is between 3.0 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.20 g of Triclofos Sodium. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.178%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Triclofos 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).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Triclofos Sodium according to Method 1, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.3 g of Triclofos Sodium, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20°C for 30 minutes. Perform the test with these solutions, using a solution obtained in the same manner with 5 mL of water as the blank, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Determine the absorbances, A_T and A_S, of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the content of the free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of the free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of the sample

Loss on drying <*2.41*> Not more than 5.0% (1 g, in vacuum, 100°C, 3 hours).

Assay **(1)** Triclofos sodium—Weigh accurately about 0.2 g of Triclofos Sodium, previously dried, place in a Kjeldhal flask, add 2 mL of sulfuric acid and 2.5 mL of nitric acid, and heat until brown gas are not evolved. After cooling, add 1 mL of nitric acid, heat until white fumes are produced, and cool. Repeat this procedure until the solution becomes colorless. Transfer this solution to a flask using 150 mL of water, add 50 mL of molybdenum (III) oxide-citric acid TS, heat gently to boil, add gradually 25 mL of quinoline TS with stirring, and heat on a water bath for 5 minutes. After cooling, filter the precipitate, and wash repeatedly with water until the washing does not indicate acidity. Transfer the precipitate to a flask using 100 mL of water, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, dissolve, and titrate <*2.50*> with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from purple to yellow (indicator: 3 drops of phenolphthalein-thymol blue TS). Perform a blank determination.

$$\text{Each mL of 0.5 mol/L sodium hydroxide VS} = 4.834 \text{ mg of } C_2H_3Cl_3NaO_4P$$

(2) Chlorine—Weigh accurately about 10 mg of Triclofos Sodium, previously dried, perform the test according to the procedure of determination for chlorine as directed under Oxygen Flask Combustion Method <*1.06*>, using 1 mL of sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Triclofos Sodium Syrup

Monosodium Trichloroethyl Phosphate Syrup

トリクロホスナトリウムシロップ

Triclofos Sodium Syrup contains not less than 90.0% and not more than 110.0% of the labeled amount of triclofos sodium ($C_2H_3Cl_3NaO_4P$: 251.37).

Method of preparation Prepare as directed under Syrups, with Triclofos Sodium.

Identification **(1)** Weigh a portion of Triclofos Sodium Syrup, equivalent to 0.25 g of Triclofos Sodium according to the labeled amount, add 40 mL of water, shake well, add 5 mL of diluted sulfuric acid (3 in 50), and extract with 25 mL of 3-methyl-1-butanol. Take 5 mL of the extract, evaporate on a water bath to dryness, and add 1 mL of diluted sulfuric acid (1 in 2) and 1 mL of a solution of potassium permanganate (1 in 20) to the residue. Heat in a water bath for 5 minutes, add 7 mL of water, and then add a solution of oxalic acid dihydrate (1 in 20) until the color of the solution disappears. To 1 mL of this solution add 1 mL of pyridine and 1 mL of a solution of sodium hydroxide (1 in 5), and heat in a water bath, while shaking, for 1 minute: a light red color develops in the pyridine layer.

(2) Take 10 mL of the extract obtained in (1), evaporate on a water bath to dryness, add 1 g of anhydrous sodium carbonate to the residue, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride. The remainder of the filtrate responds to the Qualitative Tests <*1.09*> (1) for chloride and to the Qualitative Tests <*1.09*> for phosphate.

pH <*2.54*> 6.0 – 6.5

Assay Weigh accurately a portion of Triclofos Sodium Syrup, equivalent to 0.13 g of Triclofos Sodium according to the labeled amount, add 15 mL of water, 1 mL of sodium hydroxide TS and 15 mL of diethyl ether, shake for 1 minute, and separate the water layer. Wash the diethyl ether layer with 1 mL of water, and combine the washing with above water layer. To this solution add 2.5 mL of diluted sulfuric acid (3 in 50), and extract with four 10-mL portions of 3-methyl-1-butanol. Combine the 3-methyl-1-butanol extracts, and add 3-methyl-1-butanol to make exactly 50 mL. Measure exactly 10 mL each of this solution, and dilute with potassium hydroxide-ethanol TS. Place in a glass ampule, fire-seal, mix, and heat at 120°C for 2 hours in an autoclave. After cooling, transfer the contents to a flask, add 20 mL of diluted nitric acid (63 in 500) and exactly 25 mL of 0.02 mol/L silver nitrate VS, shake well, and titrate <*2.50*> the

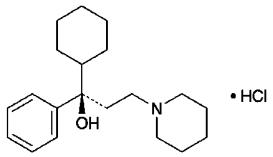
excess silver nitrate with 0.02 mol/L ammonium thiocyanate VS (indicator: 2 to 3 drops of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.02 mol/L silver nitrate VS
= 1.676 mg of $C_2H_3Cl_3NaO_4P$

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Trihexyphenidyl Hydrochloride

トリヘキシフェニジル塩酸塩



and enantiomer

$C_{20}H_{31}NO.HCl$: 337.93
(1*RS*)-1-Cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol monohydrochloride
[*52-49-3*]

Trihexyphenidyl Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{31}NO.HCl$.

Description Trihexyphenidyl Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in ethanol (95), sparingly soluble in acetic acid (100), slightly soluble in water, very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification **(1)** Dissolve 1 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool. Use this solution as the sample solution. To 5 mL of the sample solution add 1 mL of a solution of 2,4,6-trinitrophenol in chloroform (1 in 50), and shake vigorously: a yellow precipitate is formed.

(2) To 20 mL of the sample solution obtained in (1) add 2 mL of sodium hydroxide TS: a white precipitate is formed. Collect the precipitate, wash with a small amount of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the crystals so obtained melt ⟨*2.60*⟩ between 113°C and 117°C.

(3) The sample solution obtained in (1) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 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 Trihexyphenidyl Hydrochloride in 100 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Dissolve 1.5 g of Trihexyphenidyl Hydrochloride in 60 mL of water by warming on a water bath at 80°C, cool, and filter. To 40 mL of the filtrate 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, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Piperidylpropiophenone—Dissolve 0.10 g of Trihexyphenidyl Hydrochloride in 40 mL of water and 1 mL of 1 mol/L hydrochloric acid VS by warming, cool, and add water to make 100 mL. Determine the absorbance of this solution at 247 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance is not more than 0.50.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trihexyphenidyl Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 33.79 mg of $C_{20}H_{31}NO.HCl$

Containers and storage Containers—Tight containers.

Trihexyphenidyl Hydrochloride Tablets

トリヘキシフェニジル塩酸塩錠

Trihexyphenidyl Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$: 337.93).

Method of preparation Prepare as directed under Tablets, with Trihexyphenidyl Hydrochloride.

Identification **(1)** Weigh a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.1 g of Trihexyphenidyl Hydrochloride according to the labeled amount, add 30 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water by warming, cool, and use this solution as the sample solution. With 5 mL of the sample solution, proceed as directed in the Identification (1) under Trihexyphenidyl Hydrochloride.

(2) Shake a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.01 g of Trihexyphenidyl Hydrochloride according to the labeled amount, with 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.02 g of Trihexyphenidyl Hydrochloride RS in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and 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 hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots from the sample solution and the standard solution show a blue-purple color and the same *R*f value.

(3) The sample solution obtained in (1) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trihexyphenidyl Hydrochloride Tablets add 2 mL of dilute hydrochloric acid and 60 mL of water, disintegrate by vigorous shaking for 10 minutes, and warm on a water bath with occasional shaking for 10 minutes. Cool, add 2 mL of methannol, and add water to make exactly V mL of the solution contains about 20 μg of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) per ml. Centrifuge, if necessary, and use the supernatant liquid as the sample solution. Separately, dissolve about 20 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying ⟨*2.41*⟩ in the same conditions as Trihexyphenidyl Hydrochloride) in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, transfer to glass-stoppered centrifuge tubes, add exactly 10 mL of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL of chloroform, stopper tightly, shake well, and centrifuge. Pipet 10 mL each of the chloroform layers, add chloroform to make exactly 50 mL. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, respectively.

Amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$)
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of Trihexyphenidyl Hydrochlochloride RS, calculated on the dried basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Trihexyphenidyl Hydrochloride Tablets is not less than 70%.

Start the test with 1 tablet of Trihexyphenidyl Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 2.2 μg of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Trihexyphenidyl Hydrochloride RS, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution, the standard solution and the dissolution medium add exactly 1 mL of diluted acetic acid (31) (1 in 10), and immediately add 5 mL of bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS, and shake. Then, add exactly 10 mL each of dichloromethane, shake well, centrifuge, and take the dichloromethane layer. Determine the absorbances, A_T, A_S and A_B, of these dichloromethane layers at 415 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using dichloromethane as the blank.

Dissolution rate (%) with respect to the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$)
$= M_S \times (A_T - A_B)/(A_S - A_B) \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Trihexyphenidyl Hydrochloride RS
C: Labeled amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Trihexyphenidyl Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$), dissolve in 2 mL of dilute hydrochloric acid and 60 mL of water by warming on a water bath for 10 minutes with occasional shaking. After cooling, add 2 mL of methanol and water to make exactly 100 mL, and use this solution as the sample solution. Dissolve about 50 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying ⟨*2.41*⟩ in the same conditions as Trihexyphenidyl Hydrochloride), weighed accurately, in methanol, add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL each of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL each of chloroform, stopper tightly, shake thoroughly, and centrifuge. Pipet 10 mL each of the chloroform layers, and add chloroform to make exactly 50 mL. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, respectively.

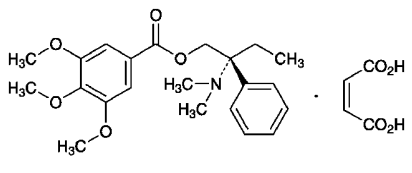
Amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of Trihexyphenidyl Hydrochloride RS, calculated on the dried basis

Containers and storage Containers—Tight containers.

Trimebutine Maleate

トリメブチンマレイン酸塩



and enantiomer

$C_{22}H_{29}NO_5.C_4H_4O_4$: 503.54
(2*RS*)-2-Dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate monomaleate
[*34140-59-5*]

Trimebutine Maleate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{22}H_{29}NO_5.C_4H_4O_4$.

Description Trimebutine Maleate occurs as white, crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), soluble in acetonitrile, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of it in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimebutine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 131 – 135°C

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Trimebutine 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) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Trimebutine Maleate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Trimebutine Maleate in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and trimebutine from the sample solution is not larger than 1/2 times the peak area of trimebutine from the standard solution, and the total area of the peaks other than maleic acid and trimebutine is not larger than the peak area of trimebutine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside 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: To 650 mL of diluted perchloric acid (17 in 20,000), previously adjusted the pH to 3.0 with a solution of ammonium acetate (1 in 1000), add 1 g of sodium 1-pentanesulfonate to dissolve. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of trimebutine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of trimebutine beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 20 mL. Confirm that the peak area of trimebutine obtained from 20 μL of this solution is equivalent to 20 to 30% of that from 20 μL of the standard solution.

System performance: Dissolve 40 mg of Trimebutine Maleate and 20 mg of imipramine hydrochloride in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7). When the procedure is run with 20μL of this solution under the above operating conditions, trimebutine and imipramine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

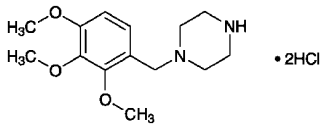
Assay Weigh accurately about 0.8 g of Trimebutine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.35 mg of $C_{22}H_{29}NO_5.C_4H_4O_4$

Containers and storage Containers—Well-closed containers.

Trimetazidine Hydrochloride

トリメタジジン塩酸塩



$C_{14}H_{22}N_2O_3.2HCl$: 339.26
1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride
[*13171-25-0*]

Trimetazidine Hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{14}H_{22}N_2O_3.2HCl$, calculated on the anhydrous basis.

Description Trimetazidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in formic acid, sparingly soluble in methanol, and slightly soluble in ethanol (99.5).

The pH of a solution of Trimetazidine Hydrochloride (1 in 20) is between 2.3 and 3.3.

Melting point: about 227°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trimetazidine Hydrochloride in 0.1 mol/L hy-

drochloric acid TS (1 in 6250) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetazidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetazidine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Trimetazidine 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—Dissolve 0.2 g of Trimetazidine Hydrochloride in 50 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 20 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than trimetazidine obtained from the sample solution is not larger than 1.5 times that of trimetazidine from the standard solution, and the total area of the peaks other than trimetazidine from the sample solution is not larger than 2.5 times the peak area of trimetazidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 2.87 g of sodium 1-heptanesulfonate in water to make 1000 mL, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10). Mix 3 volumes of this solution and 2 volumes of methanol.

Mobile phase B: Methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 50	95 → 75	5 → 25

Flow rate: Adjust the flow rate so that the retention time of trimetazidine is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of trimetazidine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of trimetazidine obtained from 10 μL of this solution is equivalent to 18 to 32% 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 the symmetry factor of the peak of trimetazidine are not less than 15,000 and not more than 1.5, respectively.

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

Water <*2.48*> Not more than 1.5% (2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of Trimetazidine Hydrochloride, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat at 90 – 100°C for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate <*2.50*> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 16.96 mg of $C_{14}H_{22}N_2O_3.2HCl$

Containers and storage Containers—Tight containers.

Trimetazidine Hydrochloride Tablets

トリメタジジン塩酸塩錠

Trimetazidine Hydrochloride Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$: 339.26).

Method of preparation Prepare as directed under Tablets, with Trimetazidine Hydrochloride.

Identification Shake a quantity of powdered Trimetazidine Hydrochloride Tablets, equivalent to 10 mg of Trimetazidine Hydrochloride according to the labeled amount, with 10 mL of a mixture of ethanol (95) and water (3:1), and filter. Evaporate the filtrate on a water bath, add 2 mL of water to the residue, and shake. To 1 mL of this solution add 1 mL of *p*-benzoquinone TS, boil gently for 2 to 3 minutes, and cool: a red color develops.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trimetazidine Hydrochloride Tablets add 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to disintegrate the tablet, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL. Centrifuge, pipet *V* mL of the supernatant liquid, equivalent to about 0.75 mg of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$), add exactly 2.5 mL of the internal

standard solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay (separately determine the water <2.48> in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of trimetazidine hydrochloride } (C_{14}H_{22}N_2O_3.2HCl) = M_S \times Q_T/Q_S \times 1/2V$$

M_S: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Trimetazidine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Trimetazidine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 3.3 μg of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$) according to the labeled amount. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of trimetazidine hydrochloride for assay (separately determine the water <2.48> in the same manner as Trimetazidine Hydrochloride), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of trimetazidine.

$$\text{Dissolution rate (\%) with respect to the labeled amount of trimetazidine hydrochloride } (C_{14}H_{22}N_2O_3.2HCl) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$) 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Assay Weigh accurately not less than 20 tablets of Trimetazidine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$), add about 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1), and treat with ultrasonic waves for 10 minutes. Then shake for 10 minutes, add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL, and centrifuge. To exactly 5 mL of the supernatant liquid add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay (separately determine the water <2.48> in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of trimetazidine to that of the internal standard.

$$\text{Amount (mg) of trimetazidine hydrochloride } (C_{14}H_{22}N_2O_3.2HCl) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside 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 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and methanol (17:3).

Flow rate: Adjust the flow rate so that the retention time of trimetazidine 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, trimetazidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

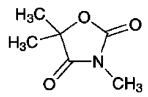
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trimetazidine to that of the internal stand-

ard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trimethadione

トリメタジオン



$C_6H_9NO_3$: 143.14
3,5,5-Trimethyl-1,3-oxazolidine-2,4-dione
[*127-48-0*]

Trimethadione, when dried, contains not less than 98.0% of $C_6H_9NO_3$.

Description Trimethadione occurs as white crystals or crystalline powder. It has a camphor-like odor.

It is very soluble in ethanol (95) and in chloroform, freely soluble in diethyl ether, and soluble in water.

Identification **(1)** To 5 mL of a solution of Trimethadione (1 in 50) add 2 mL of barium hydroxide TS: a precipitate is formed immediately.

(2) Determine the infrared absorption spectrum of a solution of Trimethadione in chloroform (1 in 50) as directed in the solution method under Infrared Spectrophotometry <*2.25*>, using a 0.1-mm fixed sodium chloride cell, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Meting point <*2.60*> 45 – 47°C

Purity Heavy metals <*1.07*>—Proceed with 2.0 g of Trimethadione 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 <*2.41*> Not more than 0.5% (1 g, silica gel, 6 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Trimethadione, previously dried, in a glass-stoppered conical flask, dissolve in 5 mL of ethanol (95), add exactly measured 50 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 15 minutes with occasional shaking. Titrate <*2.50*> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 14.31 mg of $C_6H_9NO_3$

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Trimethadione Tablets

トリメタジオン錠

Trimethadione Tabelts contain not less than 94.0% and not more than 106.0% of the labeled amount of trimethadione ($C_6H_9NO_3$: 143.14).

Method of preparation Prepare as directed under Tablets, with Trimethadione.

Identification **(1)** Weigh a portion of powdered Trimethadione Tablets, equivalent to 1 g of Trimethadione according to the labeled amount, add 10 mL of petroleum benzin, and shake frequently for 15 minutes. Decant, remove the petroleum benzin, add another 10 mL of petroleum benzin, and repeat the extraction in the same manner. To the residue add 25 mL of diethyl ether, allow to stand for 20 minutes with occasional shaking, filter, evaporate the filtrate at room temperature, and dry the residue in a desiccator (silica gel) for 6 hours: the residue melts <*2.60*> between 44°C and 47°C. Proceed with this residue as directed in the Identification (1) under Trimethadione.

(2) Determine the infrared absorption spectrum of a solution of the residue obtained in (1) in chloroform (1 in 50) in a 0.1-mm fixed sodium chloride cell, as directed in the solution method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2960 cm^{-1}, 1814 cm^{-1}, 1735 cm^{-1}, 1445 cm^{-1}, 1394 cm^{-1}, 1290 cm^{-1}, 1100 cm^{-1} and 1055 cm^{-1}.

Assay Weigh accurately and powder not less than 20 Trimethadione Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of trimethadione ($C_6H_9NO_3$), add 50 mL of ethanol (95), and boil gently for 15 minutes under a reflux condenser. Filter the warm ethanol (95) solution into a 100-mL volumetric flask through a glass filter (G4), and wash the residue with three 10-mL portions of warm ethanol (95). Combine the washings with the filtrate in the flask, cool, and add ethanol (95) to make exactly 100 mL. Pipet 25 mL of the solution into a glass-stoppered conical flask, add 25 mL of water and exactly 30 mL of 0.1 mol/L sodium hydroxide VS, stopper, allow to stand for 15 minutes with occasional shaking, and titrate <*2.50*> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

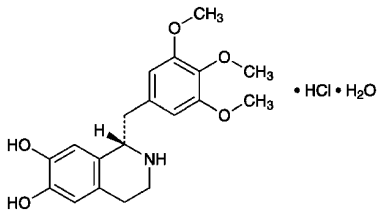
Each mL of 0.1 mol/L sodium hydroxide VS
= 14.31 mg of $C_6H_9NO_3$

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Trimetoquinol Hydrochloride Hydrate

Tretoquinol Hydrochloride

トリメトキノール塩酸塩水和物



$C_{19}H_{23}NO_5.HCl.H_2O$: 399.87
(1*S*)-1-(3,4,5-Trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol monohydrochloride monohydrate
[*18559-59-6*, anhydride]

Trimetoquinol Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of trimetoquinol hydrochloride ($C_{19}H_{23}NO_5.HCl$: 381.85), calculated on the anhydrous basis.

Description Trimetoquinol Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

Melting point: about 151°C (with decomposition, after drying in vacuum, 105°C, 4 hours).

Identification **(1)** Determine the absorption spectrum of a solution of Trimetoquinol Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetoquinol Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetoquinol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (1) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −16 – −19° (0.25 g, calculated on the anhydrous basis, water, after warming and cooling, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Trimetoquinol Hydrochloride Hydrate in 100 mL of water by warming, and cool: the pH of this solution is between 4.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Trimetoquinol Hydrochloride Hydrate in 10 mL of water by warming: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 0.5 g of Trimetoquinol Hydrochloride Hydrate. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Trimetoquinol 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).

(4) Related substances—Dissolve 50 mg of Trimetoquinol Hydrochloride Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample 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 sample solution and standard solution, as directed under Liquid Chromatography <*2.01*> 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 trimetoquinol from the sample solution is not larger than the peak area of trimetoquinol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside 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: Dissolve 2 g of potassium dihydrogen phosphate and 2 g of sodium 1-pentane sulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH between 2.8 and 3.2, and filter through a membrane filter with pore size of 0.4 μm. Add 200 mL of acetonitrile to 800 mL of the filtrate.

Flow rate: Adjust the flow rate so that the retention time of trimetoquinol is about 7 minutes.

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

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of trimetoquinol obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Trimetoquinol Hydrochloride Hydrate and 1 mg of procaine hydrochloride in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, procaine and trimetoquinol 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetoquinol is not more than 2.0%.

Water <*2.48*> 3.5 – 5.5% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trimetoquinol Hydrochloride Hydrate, dissolve in 2 mL of 0.1 mol/L hydrochloric acid VS and 70 mL of ethanol (99.5) with thorough shaking, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Calculate the consumed volume of 0.1 mol/L potassium hydroxide-

ethanol VS between the first inflection point and of the second inflection point.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 38.19 mg of $C_{19}H_{23}NO_5.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dental Triozinc Paste

歯科用トリオジンクパスタ

Dental Triozinc Paste consists of a powder containing Paraformaldehyde, Thymol, anhydrous zinc sulfate and Zinc Oxide, and a solution containing Cresol, Potash Soap and Glycerin. Suitable amounts of the two components are triturated before use.

Method of preparation

(1) The powder	
Paraformaldehyde, finely powdered	10 g
Thymol, finely powdered	3 g
Zinc Sulfate Hydrate	9 g
Zinc Oxide	82 g
To make about	100 g

Heat Zinc Sulfate Hydrate at about 250°C to obtain anhydrous zinc sulfate, cool, and pulverize to a fine powder. Mix homogeneously this powder with Thymol, Paraformaldehyde, and Zinc Oxide.

(2) The solution	
Cresol	40 g
Potash Soap	40 g
Glycerin	20 g
To make	100 g

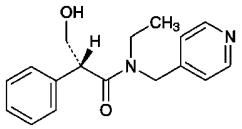
Dissolve Potash Soap in a mixture of Cresol and Glycerin.

Description The powder occurs as a fine, white powder, having a characteristic odor. The solution is a clear, yellow-brown to red-brown, viscous liquid, having the odor of cresol.

Containers and storage Containers—Tight containers.

Tropicamide

トロピカミド



and enantiomer

$C_{17}H_{20}N_2O_2$: 284.35
(2*RS*)-*N*-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide
[*1508-75-4*]

Tropicamide, when dried, contains not less than 98.5% of $C_{17}H_{20}N_2O_2$.

Description Tropicamide occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95) and in chloroform, slightly soluble in water and in diethyl ether, and practically insoluble in petroleum ether.

It dissolves in dilute hydrochloric acid.

The pH of a solution of Tropicamide (1 in 500) is between 6.5 and 8.0.

Identification **(1)** To 5 mg of Tropicamide add 0.5 mL of a solution of ammonium vanadate (V) in sulfuric acid, (1 in 200), and heat: a blue-purple color develops.

(2) Dissolve 5 mg of Tropicamide in 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. Cool, and add 2 to 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-purple color develops.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (255 nm): 166 – 180 (after drying, 5 mg, 2 mol/L hydrochloric acid TS, 200 mL).

Melting point ⟨*2.60*⟩ 96 – 99°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), 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 0.45 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95), 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.016%).

(2) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), 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, 30 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) *N*-Ethyl-γ-picolylamine—Dissolve 0.10 g of Tropicamide in 5 mL of water by heating, add 1 mL of a solution of acetaldehyde (1 in 20), and shake well. Add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS and 1 to 2 drops of sodium hydrogen carbonate TS, and shake: no blue color develops.

(4) Tropic acid—To 10 mg of Tropicamide add 5 mg of sodium borate and 7 drops of 4-dimethylaminobenzaldehyde TS, and heat in a water bath for 3 minutes. Cool in ice water, and add 5 mL of acetic anhydride: no red-purple

color develops.

Loss on drying ⟨2.41⟩ Not more than 0.30% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

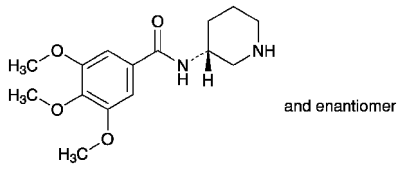
Assay Weigh accurately about 0.5 g of Tropicamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.44 mg of $C_{17}H_{20}N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Troxipide

トロキシピド



$C_{15}H_{22}N_2O_4$: 294.35
3,4,5-Trimethoxy-*N*-[(3*RS*)-piperidin-3-yl]benzamide
[*30751-05-4*]

Troxipide, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{22}N_2O_4$.

Description Troxipide occurs as a white, crystalline powder.

It is freely soluble in acetate (100), soluble in methanol, sparingly soluble in ethanol (99.5) and slightly soluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Troxipide in 1 mol/L hydrochloric acid TS (1 in 5) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Troxipide in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Troxipide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Troxipide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Troxipide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨2.60⟩ 177 – 181°C

Purity **(1)** Chloride ⟨1.03⟩—Dissolve 1.0 g of Troxipide in 30 mL of methanol, 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 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals ⟨1.07⟩—Moisten 2.0 mg of Troxipide with 1 mL of sulfuric acid, and gently heat until charred. After cooling, add 2 mL of nitric acid, carefully heat until white fumes are no longer evolved, and perform the test according to Method 2. Prepare the control solution as follows: evaporate 1 mL of sulfuric acid, 2 mL of nitric acid and 2 mL of hydrochloric acid on a water bath and then on a sand bath to dryness, and moisten the residue with 3 drops of hydrochloric acid. Proceed in the same manner 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) Related substances—Dissolve 0.20 g of Troxipide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 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 ⟨2.03⟩. Spot 5 μL each of the sample solution and 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, water, hexane and ammonia water (28) (20:20:5:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying ⟨2.41⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Troxipide, previously dried, dissolve in 40 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.44 mg of $C_{15}H_{22}N_2O_4$

Containers and storage Containers—Tight containers.

Troxipide Fine Granules

トロキシピド細粒

Troxipide Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Granules, with Troxipide.

Identification To a quantity of Troxipide Fine Granules, equivalent to 20 mg of Troxipide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir, and filter. To 4 mL of the filtrate add 0.1 mol/L hydro-

chloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Troxipide Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Troxipide Fine Granules, add 80 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide ($C_{15}H_{22}N_2O_4$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S \times Q_T/Q_S \times V/25$

M_S: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Troxipide Fine Granules is not less than 85%.

Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.1 g of troxipide ($C_{15}H_{22}N_2O_4$) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 258 nm.

Dissolution rate (%) with respect to the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 450$

M_S: Amount (mg) of Troxipide RS
M_T: Amount (mg) of sample
C: Labeled amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$) in 1 g

Particle size <*6.03*> It meets the requirements of Fine granules.

Assay Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.5 g of troxipide ($C_{15}H_{22}N_2O_4$), add 200 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and water to make 100 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of troxipide to that of the internal standard.

Amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S \times Q_T/Q_S \times 20$

M_S: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 nm).
Column: A stainless steel column 4.6 mm in inside 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 30°C.
Mobile phase: To diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydrofuran.
Flow rate: Adjust the flow rate so that the retention time of troxipide is about 7 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of standard solution under the above operating conditions, troxipide 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Troxipide Tablets

トロキシピド錠

Troxipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Tablets, with Troxipide.

Identification Weigh accurately an amount of powdered Troxipide Tablets, equivalent to 0.1 g of Troxipide according to the labeled amount, add 250 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 4 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 Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maximum between 256 nm and 260 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Troxipide Tablets add 90 mL of 0.1 mol/L hydrochloric acid TS, shake well to disintegrate, shake for another 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide ($C_{15}H_{22}N_2O_4$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S \times Q_T/Q_S \times V/25$

M_S: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Troxipide Tablets is not less than 70%.

Start the test with 1 tablet of Troxipide Tablets, withdraw not less than 20 mL of the medium at the specified minute 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, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg of troxipide ($C_{15}H_{22}N_2O_4$) according to the labeled amount, and use this solution as the sample solution. Separately weigh accurately about 20 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 258 nm.

Dissolution rate (%) with respect to the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Troxipide RS
C: Labeled amount (mg) of Troxipide ($C_{15}H_{22}N_2O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Troxipide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of troxipide ($C_{15}H_{22}N_2O_4$), add 150 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, and add 0.1 mol/L hydrochloric acid TS to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, 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 <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of troxipide to that of the internal standard.

Amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$)
$= M_S \times Q_T/Q_S \times 40$

M_S: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

Column: A stainless steel column 4.6 mm in inside 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 30°C.

Mobile phase: To 1500 mL of diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydronfuran.

Flow rate: Adjust the flow rate so that the retention time of troxipide is about 7 minutes.

System suitability—

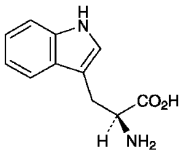
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, troxipide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Tryptophan

L-トリプトファン



$C_{11}H_{12}N_2O_2$: 204.23
(2*S*)-2-Amino-3-(indol-3-yl)propanoic acid
[*73-22-3*]

L-Tryptophan, when dried, contains not less than 98.5% of $C_{11}H_{12}N_2O_2$.

Description L-Tryptophan occurs as white to yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in formic acid, slightly soluble in water,

and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Tryptophan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -30.0 – $-33.0°$ Weigh accurately about 0.25 g of L-Tryptophan, previously dried, and dissolve in 20 mL of water by warming. After cooling, add water to make exactly 25 mL, and determine the optical rotation of the solution in a 100-mm cell.

pH ⟨*2.54*⟩ Dissolve 1.0 g in 100 mL of water by warming, and cool: the pH of this solution is between 5.4 and 6.4.

Purity **(1)** Clarity of solution—Dissolve 0.20 g of L-Tryptophan in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of L-Tryptophan in 6 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 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Tryptophan in 40 mL of water and 1 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 with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Tryptophan. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Tryptophan 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).

(6) Arsenic ⟨*1.11*⟩—Dissolve 1.0 g of L-Tryptophan in 3 mL of 1 mol/L hydrochloric acid TS and 2 mL of water by heating, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Tryptophan in 1 mL of 1 mol/L hydrochloric acid TS, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 ⟨*2.03*⟩. Spot 5 μL each of the sample solution and 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 evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of L-Tryptophan, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

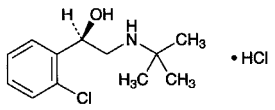
Each mL of 0.1 mol/L perchloric acid VS
= 20.42 mg of $C_{11}H_{12}N_2O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tulobuterol Hydrochloride

ツロブテロール塩酸塩



and enantiomer

$C_{12}H_{18}ClNO.HCl$: 264.19

(1*RS*)-1-(2-Chlorophenyl)-2-(1,1-dimethylethyl)aminoethanol monohydrochloride

[*56776-01-3*]

Tulobuterol Hydrochloride, when dried, contains not less than 98.5% of $C_{12}H_{18}ClNO.HCl$.

Description Tulobuterol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in water, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

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

Melting point: about 163°C.

Identification **(1)** Determine the absorption spectrum of a solution of Tulobuterol Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tulobuterol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spctra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tulobuterol Hydrochloride (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

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

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Tulobuterol 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) Related substances—Dissolve 0.30 g of Tulobuterol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol 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 ⟨*2.03*⟩. Use a

plate previously developed with the upper-layer of a mixture of ethyl acetate and ammonia solution (28) (200:9) to the top of the plate and air-dried. Spot 5 μL each of the sample solution and 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 ethyl acetate and ammonia solution (28) (200:9) 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 starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tulobuterol Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ 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
= 26.42 mg of $C_{12}H_{18}ClNO.HCl$

Containers and storage Containers—Tight containers.

Turpentine Oil

Oleum Terebinthinae

テレビン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.465 – 1.478

Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 0.860 – 0.875

Purity **(1)** Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

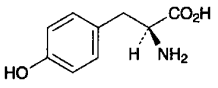
Distilling range ⟨*2.57*⟩ 150 – 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

L-Tyrosine

L-チロシン



$C_9H_{11}NO_3$: 181.19
(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoic acid
[*60-18-4*]

L-Tyrosine, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_{11}NO_3$.

Description L-Tyrosine occurs as white crystals or a crystalline powder.

It is freely soluble in formic acid, and practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in ammonia TS.

Identification **(1)** Determine the absorption spectrum of a solution of L-Tyrosine in 0.1 mol/L hydrochloric acid (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of L-Tyrosine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −10.5 – −12.5° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Tyrosine in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of L-Tyrosine in 12 mL of dilute nitric acid and 20 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 12 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Tyrosine in 5 mL of dilute hydrochloric acid, and add water to make 45 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 5 mL of dilute hydrochloric acid and water to make 45 mL. To the test solution and the control solution add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Tyrosine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Tyrosine 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).

(6) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Tyrosine according to Method 3, 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 0.20 g of L-Tyrosine in 10 mL of diluted ammonia solution (28) (1 in 2), add water to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, 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 Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

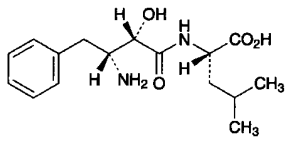
Assay Weigh accurately about 0.18 g of L-Tyrosine previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.12 mg of $C_9H_{11}NO_3$

Containers and storage Containers—Tight containers.

Ubenimex

ウベニメクス



$C_{16}H_{24}N_2O_4$: 308.37
(2*S*)-2-[(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoylamino]-4-methylpentanoic acid
[*58970-76-6*]

Ubenimex, when dried, contains not less than 98.5% and not more than 101.0% of $C_{16}H_{24}N_2O_4$.

Description Ubenimex occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in ethanol (99.5).

It dissolves in 1 mol/L hydrochloric acid TS.

Melting point: about 230°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ubenimex (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −15.5 – −17.5° (after drying, 0.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ubenimex 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 30 mg of Ubenimex in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase A to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than ubenimex obtained from the sample solution is not larger than 1/2 times the peak area of ubenimex from the standard solution. Furthermore, the total area of the peaks other than ubenimex is not larger than the peak area of ubenimex from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside 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: A mixture of diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) and acetonitrile for liquid chromatography (17:3).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) (2:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	100	0
20 – 60	100 → 0	0 → 100
60 – 70	0	100

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 14 minutes.

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

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 ubenimex obtained from 20 μL of this solution is equivalent to 7 to 13% of that 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 ubenimex are not less than 5000 and not more than 2.0, respectively.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ubenimex, previously dried, dissolve in 60 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.84 mg of $C_{16}H_{24}N_2O_4$

Containers and storage Containers—Tight containers.

Ubenimex Capsules

ウベニメクスカプセル

Ubenimex Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$: 308.37).

Method of preparation Prepare as directed under Capsules, with Ubenimex.

Identification To a quantity of the contents of Ubenimex Capsules, equivalent to 25 mg of Ubenimex according to the labeled amount, add water to make 50 mL, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Ubenimex Capsules add 30 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 50 mL. Centrifuge this solution and filter the supernatant liquid 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, equivalent to about 3 mg of ubenimex ($C_{16}H_{24}N_2O_4$), add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, 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 ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ubenimex to that of the internal standard.

Amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4$)
$= M_S \times Q_T/Q_S \times 1/V \times 15/2$

M_S: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of water and acetonitrile (7:3) (1 in 2000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ubenimex 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 2.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ubenimex Capsules is not less than 70%.

Start the test with 1 capsule of Ubenimex Capsules, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add a mixture of water and acetonitrile (7:3) to make exactly V' mL so that each mL contains about 11 μg of ubenimex ($C_{16}H_{24}N_2O_4$) according to labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ubenimex for assay, previously dried in vacume at 80°C for 4 hours, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of ubenimex in each solution.

Dissolution rate (%) with respect to the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of ubenimex for assay
C: Labeled amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4$) in 1 capsule

Operating conditions—

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 Ubenimex are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Ubenimex is not more than 2.0%.

Assay To 10 Ubenimex capsules add 140 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 200 mL. Centrifuge this solution, and filter. Discard the first 20 mL of the filtrate, pipet a volume of the subsequent filtrate, equivalent to about 7.5 mg of ubenimex ($C_{16}H_{24}N_2O_4$), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, dissolve in a mixture of water and acetonitrile (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, and a mixture of water and acetonitrile (7:3) to make 50 mL, 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 <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ubenimex to that of the internal standard.

$$\text{Amount (mg) of ubenimex } (C_{16}H_{24}N_2O_4) = M_S \times Q_T/Q_S \times 1/4$$

M_S: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in mixture of water and acetonitrile (7:3) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column 4.6 mm in inside 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 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 100) and acetonitrile for liquid chromatography (83:17).

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 8 minutes.

System suitability—

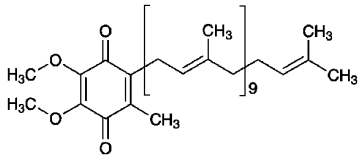
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ubenimex and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ubidecarenone

ユビデカレノン



$C_{59}H_{90}O_4$: 863.34

(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*,38*E*)-2-(3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaen-1-yl)-5,6-dimethoxy-3-methyl-1,4-benzoquinone

[*303-98-0*]

Ubidecarenone contains not less than 98.0% of $C_{59}H_{90}O_4$, calculated on the anhydrous basis.

Description Ubidecarenone occurs as a yellow to orange crystalline powder. It is odorless and has no taste.

It is soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed and colored by light.

Melting point: about 48°C.

Identification **(1)** Dissolve 0.05 g of Ubidecarenone in 1 mL of diethyl ether, and add 10 mL of ethanol (99.5). To 2 mL of this solution add 3 mL of ethanol (99.5) and 2 mL of dimethyl malonate, then add dropwise 1 mL of a solution of potassium hydroxide (1 in 5), and mix: a blue color appears.

(2) Determine the infrared absorption spectrum of Ubidecarenone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ubidecarenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Ubidecarenone 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 0.05 g of Ubidecarenone in 50 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling use this solution as the sample solution. To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 ubidecarenone from the sample solution is not larger than the peak area of ubidecarenone from 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 ubidecarenone obtained from 5 μL of the standard solution is between 20 mm and 40 mm.

Time span of measurement: About 2 times of the retention time of ubidecarenone beginning after the solvent peak.

Water ⟨*2.48*⟩ Not more than 0.20% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the water ⟨*2.48*⟩ in the same manner as Ubidecarenone) dissolve each in 40 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling add ethanol (99.5) to make exactly 50 mL each, and use these solutions as the sample solution and standard solution. Perform the test with exact 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine peak areas, A_T and A_S, of ubidecarenone of these solutions.

$$\text{Amount (mg) of } C_{59}H_{90}O_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ubidecarenone RS, calculated on the anhydrous basis

Operating conditions—

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

Column: A stainless steel column about 5 mm in inside 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 methanol and ethanol (99.5) (13:7).

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

Selection of column: Dissolve 0.01 g each of Ubidecarenone and ubiquinone–9 in 20 mL of ethanol (99.5) by warming at about 50°C for 2 minutes. After cooling, proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ubiquinone-9 and ubidecarenone in this order with the resolution between these peaks being not less than 4.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak areas of ubidecarenone is not more than 0.8%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ulinastatin

ウリナスタチン

Ulinastatin is a solution of a glycoprotein having trypsin inhibiting activity, which is separated and purified from human urine.

It contains ulinastatin of not less than 45,000 Units per mL and not less than 2500 Units per mg protein.

Description Ulinastatin occurs as a light brown to brown, clear liquid.

Identification **(1)** Dilute a suitable amount of Ulinastatin with water to make a solution containing 4000 Units of ulinastatin per mL. To 1 mL of this solution add 1 mL of a solution of phenol (1 in 20), then carefully add 5 mL of sulfuric acid, and mix: an orange to red-orange color develops.

(2) Dilute a suitable quantity of Ulinastatin with water to make a solution containing 2000 units per mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dilute a suitable amount of Ulinastatin with pH 7.8 2,2′,2″-nitrilotrisethanol buffer solution to make a solution containing 500 Units of ulinastatin per mL, and use this solution as the sample solution. Use the same buffer solution as the control solution. To 0.1 mL each of the sample solution and the control solution add 1.6 mL of the buffer solution and 0.2 mL of trypsin TS for test of ulinastatin, mix, and allow them to stand in a water bath at 25°C for 1 minute. Then add 1 mL of *N*-α-benzoyl-L-arginine-4-nitroanilide TS, mix, and allow them to stand at 25°C for 2 minutes: the solution obtained with the sample solution develops no color while that obtained with the control solution develops a yellow color.

(4) To 1.5 g of Powdered Agar add 100 mL of pH 8.4 boric acid-sodium hydroxide buffer solution, dissolve by warming in a water bath, then pour immediately into a Petri dish placed horizontally so that the agar layer is about 2 mm in thickness. After the agar becomes hard, bore two wells about 2.5 mm in diameter with a separation of 6 mm from each other. In one of the wells place 10 μL of a solution of Ulinastatin containing 500 Units per mL in pH 8.4 boric acid-sodium hydroxide buffer solution, and in the other well place 10 μL of anti-ulinastatin rabbit serum, cover the dish to avoid drying of the agar, and allow to stand for overnight at a room temperature: a clear precipitin line appears between the wells.

pH ⟨*2.54*⟩ 6.0 – 8.0

Specific activity When calculated from the results obtained by the Assay and the following method, the specific activity is not less than 2500 Units per 1 mg protein.

(i) Sample solution—To an exactly measured volume of Ulinastatin, equivalent to about 10,000 Units according to the labeled amount, add water to make exactly 20 mL.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin for test of ulinastatin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μg of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 mL each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in internal diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin's TS (1 in 2), mix, and warm in the water bath for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution obtained

in the same manner with 0.5 mL 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 calculate the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of protein per mL of Ulinastatin.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 10 mL of Ulinastatin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 1 ppm).

(2) Related substances—To a suitable volume of Ulinastatin add water to make a solution containing exactly 12,500 Units per mL, and use this solution as the sample stock solution. To exactly 0.25 mL of the sample stock solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the sample solution. Separately, to exactly 1 mL of the sample stock solution add water to make exactly 100 mL. To exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the standard solution. Perform the following test with the sample solution and the standard solution: the bands other than the principal band obtained from the sample solution are not more intense than the band from the standard solution in the electrophoretogram.

(i) Tris buffer solution for polyacrylamide gel electrophoresis A Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(ii) Tris buffer solution for polyacrylamide gel electrophoresis B Dissolve 6.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(iii) Tris buffer solution for polyacrylamide gel electrophoresis C Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 1000 mL.

(iv) Acrylamide solution for polyacrylamide gel electrophoresis Dissolve 30 g of acrylamide and 0.8 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL.

(v) Gel for separation Mix gently 15 mL of tris buffer solution for polyacrylamide gel electrophoresis A, 20 mL of acrylamide solution for polyacrylamide gel electrophoresis, 24.5 mL of water, 0.022 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, 0.32 mL of 10% ammonium peroxodisulfate TS and 0.3 mL of 1 mol/L sodium sulfite TS, pour into a plate for slab gel preparation, then cover the gel mixture with a layer of water, and allow to set for 1 hour.

(vi) Gel for concentration Remove the water layer on the gel for separation, and pour a mixture of 2.5 mL of tris buffer solution for polyacrylamide gel electrophoresis B, 2.66 mL of acrylamide solution for polyacrylamide gel electrophoresis, 14.6 mL of water, 0.01 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, 0.2 mL of 10% ammonium peroxodisulfate TS and 0.04 mL of 1 mol/L sodium sulfite TS on the gel. Then position a plastic sample well former so that the height of the gel for concentration is about 15 mm, and allow to set for 2 hours.

(vii) Procedure

Electrophoresis—Set the gel in an apparatus for slab gel electrophoresis, and fill the upper and lower reservoirs with tris buffer solution for polyacrylamide gel electrophoresis C. Introduce carefully 10 μL each of the sample solution and standard solution into the wells using a different well for each solution, and allow electrophoresis to proceed using the electrode of the lower reservoir as the anode. Switch off the power supply when the bromophenol blue band has migrated to about 10 mm from the bottom of the gel.

Staining—Dissolve 2.0 g of Coomassie brilliant blue R-250 in a mixture of 400 mL of methanol and 100 mL of acetic acid (100), add water to make 1000 mL, and use this solution as the staining solution. Stain the gel for 2 hours in the staining solution warmed to 40°C.

Decolorization—To 100 mL of methanol and 75 mL of acetic acid (100) add water to make 1000 mL, and use this solution as the rinsing solution. Immerse the gel removed from the staining solution in the rinsing solution to decolorise.

(3) Kallidinogenase—Dilute a suitable volume of Ulinastatin with water so that each mL of the solution contains about 50,000 Units, and use this solution as the sample solution. Take exactly 0.4 mL of the sample solution into a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in a water bath at 37 ± 0.2°C for 5 minutes. Add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, allow the tube to stand in the water bath of 37 ± 0.2°C for exactly 30 minutes, then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, and use this solution as the test solution. Separately, take exactly 0.4 mL of the sample solution in a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in the water bath of 37 ± 0.2°C for 35 minutes. Then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, and use this solution as the control solution. Determine the absorbances of the test solution and the control solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank, and calculate the difference between them: the difference is not more than 0.050.

Molecular mass Dilute a suitable volume of Ulinastatin with the mobile phase so that each mL of the solution contains about 6500 Units, and use this solution as the sample solution. Separately, dissolve 1.0 mg each of γ-globulin (mol. mass: 160,000), bovine serum albumin for test of ulinastatin (mol. mass: 67,000), and myoglobin (mol. mass: 17,000) in about 1 mL of the mobile phase, and use this solution as the molecular mass reference solution. Perform the test with 50 μL each of the sample solution and molecular mass reference solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Prepare a calibration curve by plotting the logarithm of molecular masses on the vertical axis and the retention times (min) of the molecular mass reference substances on the horizontal axis, and determine the molecular mass of the sample using the calibration curve and the retention time obtained with the sample solution: the molecular mass is 67,000 ± 5000.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7 mm in inside diameter and about 60 cm in length, packed with porous silica

gel for liquid chromatography (10 – 12 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 16.33 g of potassium dihydrogenphosphate and 124.15 g of ethylene glycol in water to make 1000 mL. If necessary, adjust to pH 4.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of bovine serum albumin is about 36 minutes.

Selection of column: Proceed with 50 μL of the molecular mass reference solution according to the above operating conditions, and calculate the resolution. Use a column from which γ-globulin, bovine serum albumin and myoglobin are eluted in this order with the resolution between their peaks being not less than 1.5, respectively.

Antigenicity Dilute a suitable volume of Ulinastatin with isotonic sodium chloride solution so that each mL of the solution contains 15,000 Units, and use this solution as the sample solution. Inject 0.10 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 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 sample solution intravenously into each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into 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 into 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 none of the signs mentioned above, and all the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

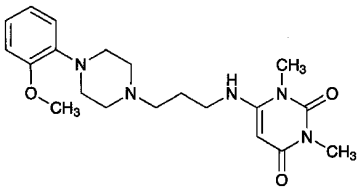
Toxicity Inject intravenously 0.50 mL of Ulinastatin into each of five well-fed, healthy albino mice weighing 18 to 25 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 5 albino mice weighing 19 to 21 g: all the animals survive for 48 hours.

Assay Measure exactly a suitable volume of Ulinastatin, dilute with 2,2′,2″-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains about 150 Units according to the labeled amount, and use this solution as the sample solution. Separately, dilute a suitable volume of Ulinastatin RS with 2,2′,2″-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains exactly 300, 200, 100, 50 or 0 Units, and use these solutions as the standard solutions. 2,2′,2″-Nitrilotrisethanol buffer solution, pH 7.8 and *N*-α-benzoyl-L-arginine-4-nitroanilide TS are warmed in a water bath of 25 ± 1°C for use as described below. Take exactly 0.1 mL each of the sample solution and the standard solutions in test tubes, add exactly 1.6 mL of 2,2′,2″-nitrilotrisethanol buffer solution, pH 7.8 mix, and put the tubes in the water bath of 25 ± 1°C. One minute after addition of the buffer solution add exactly 0.2 mL of ice-cooled trypsin TS for test of ulinastatin, mix, and put the tubes again in the water bath. One minute later add exactly 1 mL of *N*-α-benzoyl-L-arginine-4-nitroanilide TS, mix, and then put the tubes in the water bath. Exactly 2 minutes later add exactly 0.1 mL of diluted acetic acid (100) (1 in 2) to stop the enzyme reaction, and determine the absorbances of the solutions so obtained at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank. Prepare a calibration curve using the absorbances obtained with the standard solutions, and calculate ulinastatin Units in the sample solution from its absorbance by using this curve.

Containers and storage Containers—Tight containers.
Storage—Not exceeding at −20°C.

Urapidil

ウラピジル



$C_{20}H_{29}N_5O_3$: 387.48
6-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propylamino}-1,3-dimethyluracil
[*34661-75-1*]

Urapidil, when dried, contains not less than 98.0% and not more than 101.0% of $C_{20}H_{29}N_5O_3$.

Description Urapidil occurs as white to pale yellowish, white, crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Urapidil in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Urapidil as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 156 – 161°C

Purity (1) Chloride <*1.03*>—Dissolve 3.0 g of Urapidil in 40 mL of acetone and 6 mL of dilute nitric acid, add water to make 50 mL, and 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 0.003%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Urapidil 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 40 mg of Urapidil in 5

mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, ethanol (95) and ammonia water (28) (22:13:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot appears not more than one and it is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

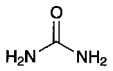
Assay Weigh accurately about 70 mg of Urapidil, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.92 mg of $C_{20}H_{29}N_5O_3$

Containers and storage Containers—Tight containers.

Urea

尿素



CH_4N_2O: 60.06
Urea
[*57-13-6*]

Urea contains not less than 99.0% of CH_4N_2O.

Description Urea occurs as colorless to white crystals or crystalline powder. It is odorless, and has a cooling, saline taste.

It is very soluble in water, freely soluble in boiling ethanol (95), soluble in ethanol (95), and very slightly soluble in diethyl ether.

A solution of Urea (1 in 100) is neutral.

Identification **(1)** Heat 0.5 g of Urea: it liquefies and the odor of ammonia is perceptible. Continue heating until the liquid becomes turbid, then cool. Dissolve the resulting lump in a mixture of 10 mL of water and 2 mL of sodium hydroxide TS, and add 1 drop of copper (II) sulfate TS: a reddish purple color develops.

(2) Dissolve 0.1 g of Urea in 1 mL of water, and add 1 mL of nitric acid: a white, crystalline precipitate is formed.

Melting point <*2.60*> 132.5 – 134.5°C

Purity **(1)** Chloride <*1.03*>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(2) Sulfate <*1.14*>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Urea 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) Ethanol-insoluble substances—Dissolve 5.0 g of Urea in 50 mL of warm ethanol (95), filter through a tared glass filter (G4), wash the residue with 20 mL of warm ethanol (95), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Urea, dissolve in water, and make exactly 200 mL. Measure exactly 5 mL of this solution into a Kjeldahl flask, and proceed as directed under Nitrogen Determination <*1.08*>.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.3003 mg of CH_4N_2O

Containers and storage Containers—Well-closed containers.

Urokinase

ウロキナーゼ

[*9010-53-1*]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000.

It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

Description Urokinase is a clear and colorless liquid.

The pH is between 5.5 and 7.5.

Identification **(1)** Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop 10 μL of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for overnight: lysis circle is appeared.

(2) Dissolve 1.0 g of Powdered Agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish until the height come to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well place separately 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and 10 μL of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 mL of Urokinase 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) Blood group substances—Dilute Urokinase with iso-

tonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μL each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add 25 μL of the sample solution into the six wells on the first lane and 25 μL of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add 50 μL of A-type erythrocyte suspension, mix, allow to stand for 2 hours, and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes.

Perform the same test by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High-molecular mass urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas of two peaks eluted closely at about 35 minutes having smaller retention time, A_a, and larger retention time, A_b, by the automatic integration method: the value, $A_a/(A_a + A_b)$, is not less than 0.85.

Operating conditions—

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 365 nm, fluorescence wavelength: 460 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 to 12 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Reaction coil: A stainless steel column 0.25 mm in inside diameter and 150 cm in length.

Reaction coil temperature: 37°C.

Mobile phase: Gelatin-phosphate buffer solution.

Flow rate of mobile phase: 0.5 mL per minute.

Reaction reagent: 7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS.

Flow rate of reaction reagent: 0.75 mL per minute.

Selection of column: Adjust the pH of Urokinase to 7.5 with sodium hydroxide TS, allow to stand at 37°C for over 24 hours, and add gelatin-phosphate buffer solution to make the solution containing 20,000 Units per mL. Proceed with 100 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of high molecular mass urokinase (mol. wt.: 54,000) and low molecular mass urokinase (mol. wt.: 33,000) in this order with the resolution between these peaks being not less than 1.0.

Assay **(1)** Urokinase—Pipet 1 mL of Urokinase, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the sample solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ampoule of High-Molecular Mass Urokinase RS to dissolve, pipet 1 mL of this solution, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the standard solution. Place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two silicon-coated test tubes about 10 mm in inside diameter, warm them in a water bath at 35 ± 0.2°C for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at 35 ± 0.2°C for exactly 30 minutes, then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, A_T and A_S, of these solutions at 405 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank. Separately place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two test tubes, add 0.50 mL of diluted acetic acid (100) (2 in 5), and 0.50 mL each of the sample solution and the standard solution. Determine the absorbances, A_{T0} and A_{S0}, of these solutions at 405 nm as the same manner, using water as the blank.

$$\text{Amount (Units) of Urokinase} = (A_T - A_{T0})/(A_S - A_{S0}) \times a \times b$$

a: Amount (Units) of urokinase in 1 mL of the standard solution

b: Total volume (mL) of the sample solution

(2) Protein—Measure exactly a volume of Urokinase, equivalent to about 15 mg of protein, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩.

$$\text{Each mL of 0.005 mol/L sulfuric acid VS} = 0.8754 \text{ mg of protein}$$

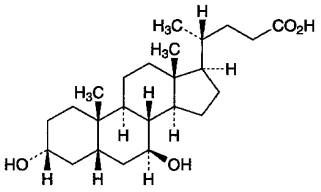
Containers and storage Containers—Tight containers.

Storage—Not exceeding −20°C.

Ursodeoxycholic Acid

Ursodesoxycholic Acid

ウルソデオキシコール酸



$C_{24}H_{40}O_4$: 392.57
3α,7β-Dihydroxy-5β-cholan-24-oic acid
[*128-13-2*]

Ursodeoxycholic Acid, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{40}O_4$.

Description Ursodeoxycholic Acid occurs as a white crystal or powder, with bitter taste.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Ursodeoxycholic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +59.0 – +62.0° (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

Melting point ⟨*2.60*⟩ 200 – 204°C

Purity **(1)** Sulfate ⟨*1.14*⟩—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100), add water to make 200 mL, and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 40 mL of the sample solution 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 by adding 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(2) Heavy metal ⟨*1.07*⟩—Proceed with 1.0 g of Ursodeoxycholic 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).

(3) Barium—To 2.0 g of Ursodeoxychollic Acid add 100 mL of water and 2 mL of hydrochloric acid, boil for 2 minutes, allow it to cool, filter, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is appeared.

(4) Related substances—Dissolve 0.10 g of Ursodeoxycholic Acid in 1 mL of methanol, add acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add acetone to make exactly 20 mL, and use these solutions as the standard solution (A) and the standard solution (B), respectively. Separately, dissolve 50 mg of chenodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, add acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution (1). Furthermore, dissolve 25 mg of lithocholic acid for thin-layer chromatography in 5 mL of methanol, and add acetone to make exactly 50 mL. Pipet 2 mL of this solution, and add acetone to make exactly 20 mL. Pipet 2 mL of this solution, add acetone 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 ⟨*2.03*⟩. Spot 10 μL each of the sample solution, standard solutions (1), (2), (A) and (B) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately spray evenly the solution which was prepared by dissolving 5 g of phosphomolybdic acid *n*-hydrate in about 50 mL of ethanol (99.5), to which 5 mL of sulfuric acid is dropped in and add ethanol (99.5) to make 100 mL, and heat at 120°C for 3 to 5 minutes: the spots from the sample solution corresponding to the spots obtained from the standard solution (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), the spots other than the principal spot and other than those mentioned above from the sample solution are not intense than the spots obtained from the standard solution (B), and the total amount of the spots other than the principal spot and other than those mentioned above from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (A) and (B), is not more than 0.25%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Containers and storage Containers—Well-closed containers.

Ursodeoxycholic Acid Granules

ウルソデオキシコール酸顆粒

Ursodeoxycholic Acid Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Granules, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic

Acid Granules, equivalent to 20 mg of Ursodeoxycholic Acid according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid 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 <2.03>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, immediately splay evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (99.5) (1 in 5), and heat at 120°C for 3 to 5 minutes: the principle spot obtained from the sample solution and the spot from the standard solution show a blue color and the same *R*f value.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Ursodeoxycholic Acid Granules is not less than 80%.

Start the test with an accurately weigh amount of Ursodeoxycholic Acid Granules, equivalent to about 50 mg of ursodeoxycholic acid ($C_{24}H_{40}O_4$) according to labeled amount, withdraw not less than 20 mL of the medium at the specified minute 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, use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ursodeoxycholic acid of each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 225$

M_S: Amount (mg) of ursodeoxycholic acid for assay
M_T: Amount (g) of Ursodeoxycholic Acid Glanules
C: Labeled amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$) in 1 g

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 of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

Assay Weigh accurately an amount of powdered Ursodeoxycholic Acid Granules, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside 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 diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).
Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid 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, ursodeoxycholic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ursodeoxycholic Acid Tablets

ウルソデオキシコール酸錠

Ursodeoxycholic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Ursodeoxycholic Acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Tablets, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic Acid Tablets, equivalent to 20 mg of Ursodeoxycholic Acid

according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid 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 <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately splay evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 3 to 5 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show a blue color and the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Ursodeoxycholic Acid Tablets and add exactly V mL of the internal standard solution so that each mL contains about 5 mg of ursodeoxycholic acid ($C_{24}H_{40}O_4$), disperse it with ultrasonic waves, then agitate to mix for 10 minutes and then centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= M_S \times Q_T/Q_S \times V/20$

M_S: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 30 minutes of a 50-mg tablet and in 45 minutes of a 100-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Ursodeoxycholic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 and pipet V mL of the subsequent filtrate. Add the dissolution medium to make exactly V' mL so that each mL contains about 56 μg of ursodeoxycholic acid ($C_{24}H_{40}O_4$) according to the labeled amount, and use the solution as the sample solution. Separately weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ursodeoxycholic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 225$

M_S: Amount (mg) of ursodeoxycholic acid for assay
C: Labeled amount (mg) of ursodeoxycholic acid in 1 tablet ($C_{24}H_{40}O_4$)

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 condition, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Ursodeoxycholic Acid Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ursodeoxycholic acid for assay

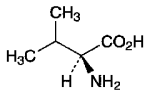
Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).
Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 6 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, ursodeoxycholic acid 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 of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Valine

L-バリン



$C_5H_{11}NO_2$: 117.15
(2*S*)-2-Amino-3-methylbutanoic acid
[*72-18-4*]

L-Valine, when dried, contains not less than 98.5% of $C_5H_{11}NO_2$.

Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly sweet taste, which becomes bitter.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Valine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +26.5 – +29.0° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of L-Valine in 20 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Valine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Valine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Valine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Valine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Valine 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).

(6) Arsenic <*1.11*>—Proceed with 1.0 g of L-Valine, prepare the test solution according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Valine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this 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 <*2.03*>. Spot 5 μL each of the sample solution and 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 evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

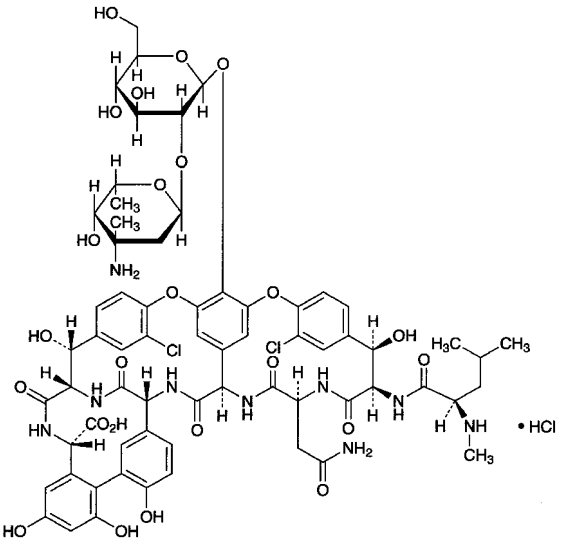
Assay Weigh accurately about 0.12 g of L-Valine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.72 mg of $C_5H_{11}NO_2$

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride

バンコマイシン塩酸塩



$C_{66}H_{75}Cl_2N_9O_{24}$.HCl: 1485.71

(1*S*,2*R*,18*R*,19*R*,22*S*,25*R*,28*R*,40*S*)-50-[3-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl-(1→2)-β-D-glucopyranosyloxy]-22-carbamoylmethyl-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[(2*R*)-4-methyl-2-(methylamino)pentanoylamino]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentaazaoctacyclo[26.14.2.2^{3,6}.2^{14,17}.1^{8,12}.1^{29,33}.0^{10,25}.0^{34,39}]pentaconta-3,5,8,10,12(50),14,16,29,31,33(49),34,36,38,45,47-pentadecaene-40-carboxylic acid monohydrochloride [*1404-93-9*]

Vancomycin Hydrochloride is the hydrochloride of a glycopeptide substance having antibacterial activity produced by the growth of *Streptomyces orientalis*.

It contains not less than 1000 μg (potency) and not more than 1200 μg (potency) per mg, calculated on the anhydrous basis. The potency of Vancomycin Hydrochloride is expressed as mass (potency) of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25).

Description Vancomycin Hydrochloride occurs as a white powder.

It is freely soluble in water, soluble in formamide, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Vancomycin Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vancomycin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vancomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Vancomycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Vancomycin Hydrochloride in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −30 – −40° (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.25 g of Vancomycin Hydrochloride in 5 mL of water is between 2.5 and 4.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Vancomycin 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 0.10 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <*2.01*> according to the following conditions. If necessary, proceed with 20 μL of the mobile phase A in the same manner to compensate for the base line. Determine each peak area by the automatic integration method: the area of each peak other than vancomycin from the sample solution is not larger than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin is not larger than 3 times of the peak area of vancomycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside 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: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (92:7:1). Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (70:29:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12	100	0
12 – 20	100 → 0	0 → 100
20 – 22	0	100

Flow rate: 1.5 mL per minute.

Time span of measurement: As long as about 2.5 times of the retention time of vancomycin beginning after the solvent peak.

System suitability—

Test for required detectability: Confirm that the peak area of vancomycin obtained from 20 μL of the standard solution is equivalent to 3 to 5% of that from 20 μL of the sample solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, heat at 65°C for 48 hours, and cool to the ordinal temperature. When the procedure is run with 20 μL of this solution under the above operating conditions, related substance 1, vancomycin and related substance 2 are eluted in this order, the resolution between the peaks of the related substance 1 and vancomycin is not less than 3, the number of theoretical plates of the peak of vancomycin is not less than 1500, and the related substance 2 is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vancomycin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (3:1)).

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.2 to 6.4 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Vancomycin Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, 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 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Vancomycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride for Injection

注射用バンコマイシン塩酸塩

Vancomycin Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25).

Method of preparation Prepare as directed under Injections, with Vancomycin Hydrochloride.

Description Vancomycin Hydrochloride for Injection occurs as white masses or a white powder.

Identification **(1)** Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 5 mg (potency) of Vancomycin Hydrochloride, in 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 279 and 283 nm.

(2) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 20 mg (potency) of Vancomycin Hydrochloride, in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water is between 2.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and colorless to pale yellow, and the absorbance of the solution, determined at 465 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.05.

(2) Related substances—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.1 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of the mobile phase A, and use this solution as the sample solution.

Proceed as directed in the Purity (2) under Vancomycin Hydrochloride.

Water ⟨*2.48*⟩ Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (3:1)).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mg (potency).

Uniformity of dosage unit ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Vancomycin Hydrochloride.

(ii) Sample solutions—Weigh accurately the contents of not less than 10 Vancomycin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled amount, and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Vasopressin Injection

バソプレシン注射液

Vasopressin Injection is an aqueous solution for injection.

It contains synthetic vasopressin or the pressor principle, vasopressin, obtained from the posterior lobe of the pituitary of healthy cattles and pigs, from which the majority of the oxytocic principle, oxytocin, has been removed.

It contains not less than 85% and not more than 120% of the labeled vasopressin Units.

Method of preparation Prepare as directed under Injections, with vasopressin prepared by synthesis or obtained from the posterior lobe of the pituitary.

Description Vasopressin Injection is a clear and colorless liquid. It is odorless or has a slight, characteristic odor.

pH: 3.0 – 4.0

Purity Oxytocic principle—When tested by the following procedure, Vasopressin Injection contains not more than 0.6 oxytocin Units for each determined 10 vasopressin Units.

(i) Standard stock solution: Dissolve 200 Units of Oxytocin RS, according to the labeled Units, in exactly 10 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(ii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that each mL of the solution contains 0.020 oxytocin Units.

(iii) Sample solution: Assume oxytocin Units as equivalent to 6/100 of the determined vasopressin Units. Dilute Vasopressin Injection with isotonic sodium chloride solution so that each mL of the resulting solution is expected to contain 0.020 oxytocin Unit.

(iv) Apparatus: Use the apparatus for the uterus contraction test, equipped with a thermostatic bath. Maintain a temperature of the bath at 37°C to 38°C with a variation of not more than 0.1°C during the course of the test. Use a 100-mL Magnus' chamber for suspending the uterus.

(v) Test animal: Use healthy, virgin and metestrus guinea pigs weighing between 175 g and 350 g. They have been bred under conditions where they have been completely isolated from the sight and smell of males since the time of weaning.

(vi) Procedure: Immerse the Magnus' chamber in the bath maintained at a constant temperature, add Locke-Ringer's solution to the chamber, and introduce oxygen into the solution at a moderate rate. Sacrifice a guinea pig by means of a blow on the head, immediately remove the uterus from the body, suspend it in the chamber, and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the mass is not changed throughout the assay. Start the assay after 15 to 30 minutes when the uterus is completely relaxed. Administer the same quantities, 0.1 to 0.5 mL, of the standard solution and the sample solution to the Magnus' chamber alternately twice with regular intervals of between 10 and 20 minutes to contract the uterus, finally administer the standard solution in a quantity which is 25% larger than the preceding doses, and measure the height of every contraction. The mean height of uterus contraction caused by the standard solution is equal to or higher than that caused by the sample solution. The height of contraction caused by the increased dose of the standard solution is distinctly higher than those caused by the preceding doses of the standard solution.

Bacterial endotoxins ⟨*4.01*⟩ Less than 15 EU/vasopressin Unit.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Test animals: Use healthy male rats weighing between 200 g and 300 g.

(ii) Standard stock solution: Dissolve 2000 Units of Vasopressin RS, according to the labeled Units, in exactly 100 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(iii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that 0.2 mL of the obtained solution causes blood pressure increases of between 35 mmHg and 60 mmHg in test animals when injected according to (vi), and designate this solution as the high-dose standard solution (S_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose standard solution (S_L).

(iv) Sample solution: Dilute an accurately measured volume of Vasopressin Injection with isotonic sodium chloride solution so that the obtained solution contains the same concentration in Units as the high-dose standard solution based on the labeled Units, and designate it as the high-dose sample solution (T_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and

designate it as the low-dose sample solution (T_L). Make the concentration ratio of S_H to S_L equal to the ratio of T_H to T_L. When the sensitivity of an animal is changed, adjust the concentration of S_H and T_H before the next set of assay is started. However, keep the same ratio of S_H to S_L and T_H to T_L as in the primary set.

(v) Dose of injection: Although 0.2 mL of each solution is usually injected, the dose of injection can be determined based from preliminary tests or experiences. Inject the same volume throughout a set of tests.

(vi) Procedure: Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body mass to anesthetize the test animals and cannulate the trachea. Under artificial respiration (about 60 strokes per minute), remove a part of the second cervical vertebra, cut off the spinal cord and destroy the brain through the foramen magnum. Insert a cannula filled with isotonic sodium chloride solution into a femoral vein. Through this cannula, inject the solution prepared by dissolving 200 heparin Units of heparin sodium in 0.1 mL of isotonic sodium chloride solution, and then immediately inject 0.3 mL of isotonic sodium chloride solution. Insert a cannula into a carotid artery, and connect the cannula to a manometer for blood pressure measurement with a vinyl tube. The cannula and the vinyl tube have previously been filled with isotonic sodium chloride solution. Inject the standard and the sample solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the isotonic solution when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 1 mmHg on the kymogram. Maintain a constant temperature between 20°C and 25°C during the assay. In advance, make four pairs from S_H, S_L, T_H, T_L as follows. Randomize the order of injection for pairs, but keep the order of injection within pairs as indicated.

Pair 1: S_H, T_L Pair 2: S_L, T_H Pair 3: T_H, S_L Pair 4: T_L, S_H

Carry out this assay using the same animals throughout a set of four pairs of observations. Perform this assay with two sets. If necessary, however, use the different animals for both sets of tests.

(vii) Calculation: Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pair 1, 2, 3 and 4 of each set, and obtain the responses y_1, y_2, y_3 and y_4, respectively. Sum up y_1, for each set to obtain Y_1, and obtain Y_2, Y_3 and Y_4 in the same way.

Units in each mL of Vasopressin Injection
= antilog M × (Units in each mL of the S_H) × b/a

$M = (IY_a/Y_b)$

$I = \log(S_H/S_L) = \log(T_H/T_L)$

$Y_a = -Y_1 + Y_2 + Y_3 - Y_4$

$Y_b = Y_1 + Y_2 + Y_3 + Y_4$

a: Volume (mL) of Vasopressin Injection sampled.

b: Total volume (mL) of the high-dose sample solution prepared by diluting with isotonic sodium chloride solution.

Compute L (P = 0.95) by the following equation, and confirm L to be 0.15 or less. If L exceeds 0.15, repeat the test, improving the conditions of the assay or increasing the number of sets until L reaches 0.15 or less.

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = \{Y_b^2/(Y_b^2 - 4fs^2t^2)\}$$

f: Number of sets

$$s^2 = \{\Sigma y^2 - (Y/f) - (Y'/4) + (Y_b^2/4f)\}/n$$

Σy^2: The sum of the squares of y_1, y_2, y_3 and y_4.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

Y': The sum of the squares of the sum of y_1, y_2, y_3 and y_4 in each set.

$$n = 3(f-1)$$

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		

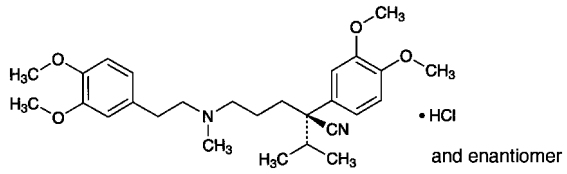
Containers and storage Containers—Hermetic containers. Storage—In a cold place, and avoid freezing.

Expiration date 36 months after preparation.

Verapamil Hydrochloride

Iproveratril Hydrochloride

ベラパミル塩酸塩



$C_{27}H_{38}N_2O_4.HCl$: 491.06

(2*RS*)-5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile monohydrochloride

[*152-11-4*]

Verapamil Hydrochloride, when dried, contains not less than 98.5% of $C_{27}H_{38}N_2O_4.HCl$.

Description Verapamil Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in methanol, in acetic acid (100) and in chloroform, soluble in ethanol (95) and in acetic anhydride, sparingly soluble in water, and practically insoluble in

diethyl ether.

Identification **(1)** To 2 mL of a solution of Verapamil Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Verapamil Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Verapamil Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 141 – 145°C.

pH <*2.54*> Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of freshly boiled and cooled water by warming, and cool: the pH of this solution is between 4.5 and 6.5.

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Verapamil 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 <*1.11*>—Prepare the test solution with 1.0 g of Verapamil Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Verapamil Hydrochloride in exactly 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet 5 mL of standard stock solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and diethylamine (17:3) to a distance of about 15 cm, air-dry the plate, heat at 110°C for 1 hour, and cool. Examine immediately after spraying evenly iron (III) chloride-iodine TS on the plate: the three spots, having more intense color in the spots other than the principal spot and the original point from the sample solution, are not more intense than the spot from the standard solution (2) in color. The remaining spots from the sample solution are not more intense than the spot from the standard solution (1) in color. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14:4:1:1), and perform the test in the same manner.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Verapamil Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> 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
= 49.11 mg of $C_{27}H_{38}N_2O_4$.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Verapamil Hydrochloride Tablets

ベラパミル塩酸塩錠

Verapamil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl: 491.06).

Method of preparation Prepare as directed under Tablets, with Verapamil Hydrochloride.

Identification **(1)** To a quantity of pulverized Verapamil Hydrochloride Tablets, equivalent to 0.2 g of Verapamil Hydrochloride according to the labeled amount, add 70 mL of 0.02 mol/L hydrochloric acid TS, and shake occasionally in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make 100 mL, and filter. To 3 mL of the filtrate add several drops of Reinecke's salt TS: a light red precipitate is formed.

(2) To 2 mL of the filtrate obtained in (1) add 0.02 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 227 nm and 231 nm, and between 276 nm and 280 nm.

Uniformity of dosage unit <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Verapamil Hydrochloride Tablets add 70 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablet by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly V mL of the subsequent filtrate, add 0.02 mol/L hydrochloric acid TS to make exactly V' so that each mL contains about 40 μg of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl), and use this solution as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of verapamil hydrochloride for assay

Assay To 10 tablets of Verapamil Hydrochloride Tablets add 140 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablets by occasional shaking in a water bath at

60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and filter. Discard the first 20 mL of the filtrate, take an exact volume of the subsequent filtrate, equivalent to about 4 mg of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl), add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of verapamil hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in 70 mL of 0.02 mol/L hydrochloric acid TS by occasional shaking in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 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 sample solution and standard solution at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

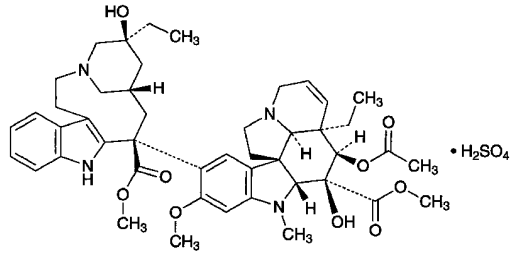
Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl) $= M_S \times A_T/A_S \times 1/25$

M_S: Amount (mg) of verapamil hydrochloride for assay

Containers and storage Containers—Tight containers.

Vinblastine Sulfate

ビンブラスチン硫酸塩



$C_{46}H_{58}N_4O_9.H_2SO_4$: 909.05

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [*143-67-9*]

Vinblastine Sulfate contains not less than 96.0% and not more than 102.0% of $C_{46}H_{58}N_4O_9.H_2SO_4$, calculated on the dried basis.

Description Vinblastine Sulfate occurs as a white to pale yellow powder.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: −28 – −35° (0.20 g calculated on the dried basis, methanol, 10 mL, 100 mm).

Identification (1) Determine the absorption spectrum of a solution of Vinblastine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vinblastine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vinblastine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Vinblastine Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vinblastine Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 15 mg of Vinblastine Sulfate 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 50 mg of Vinblastine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve about 4 mg of Vinblastine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of peak other than the main peak obtained from sample solution is not larger than 1/4 times the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 3/4 times the peak area of vinblastine 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.

Time span of measurement: About 4 times as long as the retention time of vinblastine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 100 mL. Confirm that the peak area of vinblastine obtained from 200 μL of this solution is equivalent to 1.7 to 3.3% of that from 200 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 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.5%.

Loss on drying Perform the test with about 10 mg of Vinblastine Sulfate as directed in Method 2 under the Thermal Analysis <2.52> according to the following conditions: not more than 15.0%.

Operating conditions—

Heating rate: 5°C per minute.

Temperature range: room temperature to 200°C.

Atmospheric gas: dried Nitrogen.

Flow rate of atmospheric gas: 40 mL per minute.

Assay Weigh accurately about 10 mg each of Vinblastine Sulfate and Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate),

dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of vinblastine.

Amount (mg) of $C_{46}H_{58}N_4O_9.H_2SO_4$ = $M_S \times A_T/A_S$

M_S: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column 4.6 mm in inside 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: To 7 mL of diethylamine add water to make 500 mL, and adjust the pH to 7.5 with phosphoric acid. To 380 mL of this solution add 620 mL of a mixture of methanol and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of Vinblastine Sulfate and vincristine sulfate in 25 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, vincristine and vinblastine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at not exceeding −20°C.

Vinblastine Sulfate for Injection

注射用ビンブラスチン硫酸塩

Vinblastine Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$: 909.05).

Method of preparation Prepare as directed under Injections, with Vinblastine Sulfate.

Description Vinblastine Sulfate for Injection occurs as white to pale yellow, light masses or powder.

It is freely soluble in water.

The pH of a solution (1 in 1000) is 3.5 – 5.0.

Identification Proceed as directed in the Identification (1) under Vinblastine Sulfate.

Purity Related substances—Dissolve 4 mg of Vinblastine Sulfate for Injection in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than the main peak from the sample solution is not larger than 1/2 times the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 2 times the peak area of vinblastine from the standard solution.

Operating conditions—

Perform as directed in the operating conditions in Purity (2) under Vinblastine Sulfate.

System suitability—

Perform as directed in the system suitability in Purity (2) under Vinblastine Sulfate.

Bacterial endotoxins <*4.01*> Less than 10 EU/mg.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Dissolve 1 Vinblastine Sulfate for Injection in water to make exactly V mL so that each mL contains about 0.4 mg of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

Amount (mg) of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$) = $M_S \times A_T/A_S \times 25/V$

M_S: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take an amount of Vinblastine Sulfate for Injection, equivalent to 0.10 g of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$), dissolve each content with a suitable amount of water, transfer into a 100-mL volumetric flask, wash each container with water, transfer the washings into the volumetric flask, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

Amount (mg) of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$) = $M_S \times A_T/A_S \times 10$

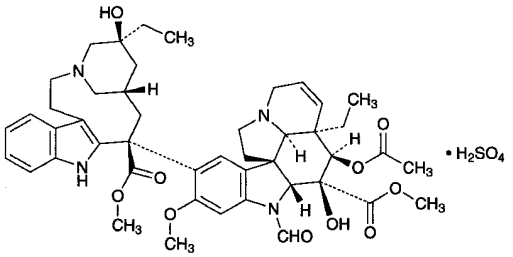
M_S: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, at 2 to 8°C.

Vincristine Sulfate

ビンクリスチン硫酸塩



$C_{46}H_{56}N_4O_{10}.H_2SO_4$: 923.04

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [*2068-78-2*]

Vincristine Sulfate contains not less than 95.0% and not more than 105.0% of $C_{46}H_{56}N_4O_{10}.H_2SO_4$, calculated on the dried basis.

Description Vincristine Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: +28.5 – +35.5° (0.2 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification **(1)** Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vincristine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vincristine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Vincristine Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 10 mg of Vincristine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 50 mg of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 10 mg of Vincristine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the peak area of desacetyl vincristine and vinblastine, having the relative retention times of about 0.9 and about 1.6 with respect to vincristine, respectively, obtained from the sample solution are not larger than 1/8 times and 3/20 times, respectively, the peak area of vincristine from the standard solution, and the area of the peak other than vincristine, desacetyl vincristine and vinblastine from the sample solution is not larger than 1/4 times the peak area of vincristine from standard solution. Furthermore, the total area of the peaks other than vincristine from the sample solution is not larger than the peak area of vincristine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

Column: A stainless steel column 4.6 mm in inside 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 A: methanol.

Mobile phase B: A mixture of water and diethylamine (197:3), adjusted the pH to 7.5 with phosphoric acid.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12	62	38
12 – 27	62 → 92	38 → 8

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

Time span of measurement: About 1.7 times as long as the retention time of vincristine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 200 mL. Confirm that the peak area of vincristine obtained from 200 μL of this solution is equivalent to 1.75 to 3.25% of that from 200 μL of the standard solution.

System performance: Dissolve 15 mg each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. When the procedure is run with 200 μL of this solution under the above operating conditions, vincristine and vinblastine 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 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.5%.

Loss on drying Perform the test with about 10 mg of Vincristine Sulfate as directed in Method 2 under Thermal Analysis <*2.52*> according to the following conditions: not

more than 12.0%.

Operating conditions—

Heating rate: 5°C per minute.

Temperature range: room temperature to 200°C.

Atmospheric gas: dried nitrogen.

Flow rate of atmospheric gas: 40 mL per minute.

Assay Weigh accurately about 10 mg each of Vincristine Sulfate and Vincristine Sulfate RS (separately determine the loss on drying in the same conditions as Vincristine Sulfate), dissolve each in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the vincristine peak areas, A_T and A_S, of both solutions.

$$\text{Amount (mg) of } C_{46}H_{56}N_4O_{10}.H_2SO_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Vincristine Sulfate RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

Column: A stainless steel column 4.6 mm in inside 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 25°C.

Mobile phase: Adjust the pH to 7.5 of a mixture of water and diethylamine (59:1) with phosphoric acid. To 300 mL of this solution add 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 7 minutes.

System suitability—

System performance: Dissolve 5 mg each of Vincristine Sulfate and vinblastine sulfate in 5 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, vincristine and vinblastine 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at not exceeding −20°C.

Vitamin A Oil

ビタミン A 油

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils.

It contains not less than 30,000 vitamin A Units per g.

It may contain suitable antioxidants.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of vitamin A.

Description Vitamin A Oil is a yellow to yellow-brown, clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.

It is decomposed upon exposure to air or light.

Identification Dissolve Vitamin A Oil, Retinol Acetate RS and Retinol Palmitate RS, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution, the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution has the same color tone and the same *R*f value with the blue spot obtained from the standard solution (1) or the standard solution (2).

Purity **(1)** Acidity—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl ether (1:1), boil gently for 10 minutes under a reflux condenser, cool, and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Rancidity—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <*2.55*>.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Capsules

ビタミン A 油カプセル

Vitamin A Oil Capsules contain not less than 90.0% and not more than 130.0% of the labeled Units of vitamin A.

Method of preparation Prepare as directed under Capsules, with Vitamin A Oil.

Description The content of Vitamin A Oil Capsules conforms to the requirements of Description under Vitamin A Oil.

Identification Proceed the test with the content of Vitamin A Oil Capsules as directed in the Identification under Vitamin A Oil.

Assay Weigh accurately 20 Vitamin A Oil Capsules, and open the capsules to take out the content. Wash the capsules well with a small amount of diethyl ether, allow the capsules to stand at ordinal temperature to vaporize the diethyl ether, and weigh accurately. Perform the test with the content as directed under Vitamin A Assay <*2.55*>, and calculate the units of vitamin A per capsule. Before applying Method 1-1, it is necessary to know which the sample is, retinol acetate or retinol palmitate.

Containers and storage Containers—Well-closed contain-

ers.

Storage—Light-resistant.

Compound Vitamin B Powder

複方ビタミン B 散

Method of preparation

Thiamine Nitrate	10 g
Riboflavin	10 g
Pyridoxine Hydrochloride	10 g
Nicotinamide	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Vitamin B Powder is orange-yellow in color. It has a slighly bitter taste.

It is slowly affected by light.

Identification **(1)** Shake 2 g of Compound Vitamin B Powder with 100 mL of water, filter, and to 5 mL of the filtrate add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and observe under ultraviolet light: the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline (thiamine).

(2) Shake 0.1 g of Compound Vitamin B Powder with 100 mL of water, and filter. Perform the following tests with the filtrate (riboflavin).

(i) The filtrate is light yellow-green in color and has an intense yellow-green fluorescence. This color and fluorescence of the solution disappears upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the filtrate, and again appears by shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(ii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, after illuminating with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake thoroughly with 5 mL of chloroform: the chloroform layer shows yellow-green fluorescence.

(3) Shake 1 g of Compound Vitamin B Powder with 100 mL of diluted ethanol (7 in 10), filter, and to 5 mL of the filtrate add 2 mL of sodium hydroxide TS and 40 mg of manganese dioxide. Heat on a water bath for 30 minutes, cool, and filter. Add 5 mL of 2-propanol to 1 mL of the filtrate, and use the solution as the sample solution. To 3 mL of the sample solution add 2 mL of bartibal buffer solution, 4 mL of 2-propanol and 2 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000) prepared when required for use: a blue color develops. To 1 mL of the sample solution add 1 mL of a saturated boric acid solution, and proceed as directed in the same manner as above: no blue color develops (pyridoxine).

(4) Shake 0.5 g of Compound Vitamin B Powder with 10 mL of ethanol (95), filter, and evaporate 1 mL of the filtrate on a water bath to dryness. Add 0.01 g of 2,4-dinitrochlorobenzen to the residue, heat gently for 5–6 seconds to fuse, and after cooling, add 4 mL of potassium hydroxide-ethanol TS: a red color develops (nicotinamide).

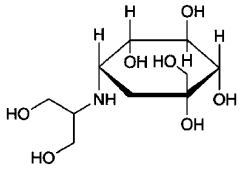
(5) Shake 1 g of Compound Vitamin B Powder with 5 mL of diluted ethanol (7 in 10), filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g each of thiamine mononitrate, riboflavin, pyridoxine hydrochloride and nicotinamide in 1 mL, 50 mL, 1 mL and 1 mL of water, respectively, and use these solutions as standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (100:50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): four spots from the sample solution show the same color tone and the same *R*f value as the corresponding spots from standard solutions (1), (2), (3) and (4).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Voglibose

ボグリボース



$C_{10}H_{21}NO_7$: 267.28

3,4-Dideoxy-4-[2-hydroxy-1-(hydroxymethyl)ethylamino]-2-*C*-(hydroxymethyl)-D-*epi*-inositol

[*83480-29-9*]

Voglibose contains not less than 99.5% and not more than 101.0% of $C_{10}H_{21}NO_7$, calculated on the anhydrous basis.

Description Voglibose occurs as white crystals or crystalline powder.

It is very slightly soluble in water, freely soluble in acetic acid (100), slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the infrared absorption spectrum of Voglibose as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Voglibose in heavy water for nuclear magnetic resonance spectroscopy (3 in 70) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits 2 double signals A at about δ 1.5 ppm, 2 double signals B at about δ 2.1 ppm, a multiple signal C at about δ 2.9 ppm, and a multiple signal D between δ 3.4 ppm and δ 3.9 ppm. The area intensity ratio of each signal, A:B:C:D, is about 1:1:1:10.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +45 – +48° (0.2 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Melting point ⟨*2.60*⟩ 163 – 168°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Voglibose according to Method 1, and perform the test. Adjust the pH of the test solution between 3.0 and 3.5 with dilute hydrochloric acid instead of dilute acetic acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Voglibose in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than voglibose obtained from sample solution is not larger than 1/5 times the peak area of voglibose from the standard solution. For the calculate of the total area, use the area of the peaks, having the relative retention time of about 1.7, about 2.0 and about 2.3 to voglibose, after multiplying by their relative response factors, 2, 2 and 2.5, respectively.

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with pentaethylenehexaaminated polyvinyl alcohol polymer bead for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 370 mL of this solution add 630 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C.

Cooling temperature: A constant temperature of about 15°C.

Flow rate of the mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of the reaction reagent: Same as the flow rate of the mobile phase.

Time span of measurement: About 2.5 times as long as the retention time of voglibose, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make 100 mL. Confirm that the peak area of voglibose obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 50 μL of the standard solution.

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 voglibose are not less than 7000 and between 0.8 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 3.0%.

Water ⟨*2.48*⟩ Not more than 0.2% (0.5 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Voglibose, dissolve in 80 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.73 mg of $C_{10}H_{21}NO_7$

Containers and storage Containers—Tight containers.

Voglibose Tablets

ボグリボース錠

Voglibose Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of voglibose ($C_{10}H_{21}NO_7$: 267.28).

Method of preparation Prepare as directed under Tablets, with Voglibose.

Identification Shake vigorously an amount of pulverized Voglibose Tablets, equivalent to 5 mg of Voglibose according to the labeled amount, with 40 mL of water, and centrifuge. Transfer the supernatant liquid to a chromatographic column [prepared by pouring 1.0 mL of strongly acidic ion-exchange resin (H type) for column chromatography (100 to 200 μm in particle diameter) into a chromatographic column 8 mm in inside diameter and 130 mm in height], and allow to flow at a rate of about 5 mL per minute. Then wash the column with 200 mL of water, and allow to flow with 10 mL

of diluted ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the effluent solution two times through a membrane filter with a pore size not exceeding 0.22 μm. Evaporate the filtrate to dryness at 50°C under reduced pressure, dissolve the residue with 0.5 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of voglibose for assay in 2 mL of the mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ammonia water (28) and water (5:3:1) to a distance of about 12 cm, air-dry the plate, and allow to stand in iodine vapors: the principal spot from the sample solution and the spot from the standard solution show a yellow-brown color, and the same *R*f value.

Uniformity of dosage unit ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Voglibose Tablets add exactly *V* mL of the mobile phase so that the solution contains about 40 μg of voglibose ($C_{10}H_{21}NO_7$) per mL, disintegrate the tablet completely by shaking, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

$$\text{Amount (g) of voglibose } (C_{10}H_{21}NO_7) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of voglibose for assay, calculated on the anhydrous basis

Assay To 20 tables of Voglibose Tablets add 80 mL of the mobile phase, and completely disintegrate by shaking. To an exact volume of the solution, equivalent to about 4 mg of voglibose ($C_{10}H_{21}NO_7$), add the mobile phase to make exactly 100 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of voglibose for assay (previously determine the water ⟨*2.48*⟩ in the same manner as Voglibose), and dissolve in the mobile phase to make exactly 25 mL. Pipet 5 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 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of voglibose.

$$\text{Amount (mg) of voglibose } (C_{10}H_{21}NO_7) = M_S \times A_T/A_S \times 1/500$$

M_S: Amount of voglibose for assay, calculated on the dried basis

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm).

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

Column temperature: A constant temperature of about 25°C.

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 300 mL of this solution add 600 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C.

Cooling temperature: A constant temperature of about 15°C.

Flow rate of mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase.

System suitability—

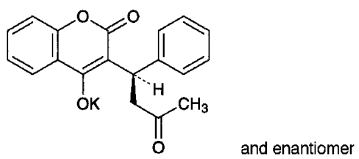
System performance: Dissolve 2 mg of voglibose for assay and 0.2 g of lactose monohydrate in 5 mL of water, and add the mobile phase to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, lactose and voglibose are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 2.0%.

Containers and storage Containers—Tight containers.

Warfarin Potassium

ワルファリンカリウム



and enantiomer

$C_{19}H_{15}KO_4$: 346.42

Monopotassium (1*RS*)-2-oxo-3-(3-oxo-1-phenylbutyl)chromen-4-olate

[*2610-86-8*]

Warfarin Potassium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{19}H_{15}KO_4$.

Description Warfarin Potassium occurs as a white, crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It dissolves in sodium hydroxide TS.

The pH of a solution prepared by dissolving 1.0 g of Warfain Potassium in 100 mL of water is 7.2 – 8.3.

It is colored to light yellow by light.

A solution of Warfarin Potassium (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Warfarin Potassium in 0.02 mol/L potassium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a slolution of Warfarin Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Warfarin Potassium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Warfarin Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Walfarin Potassium (1 in 250) responds to the Qualitative Tests <*1.09*> (1) for potassium salt.

Purity **(1)** Alkaline colored substances—Dissolve 1.0 g of Warfarin Potassium in a solution of sodium hydroxide (1 in 20) to make exactly 10 mL, and determine the absorbance at 385 nm within 15 minutes as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution of sodium hydroxide (1 in 20) as a blank: it does not exceed 0.20.

(2) Heavy metals <*1.07*>—Dissolve 2.0 g of Warfarin Potassium in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) 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, 2 mL of dilute acetic acid and ethanol (95) to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Warfarin Potassium in 100 mL of a mixture of water and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than warfarin obtained with the sample solution is not larger than 1/10 times the peak area of warfarin with the standard solution, and the total area of the peaks other than warfarin is not larger than 1/2 times the peak area of warfarin 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.

Time span of measurement: About 2 times as long as the retention time of warfarin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of water and methanol (3:1) to make exactly 20 mL. Confirm that the peak area of warfarin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: Dissolve 20 mg of propyl parahydroxybenzoate in 50 mL of methanol, and add water to make 200 mL. To 5 mL of this solution add 4 mL of a solution of Warfarin Potassium in the mixture of water and methanol (3:1) (1 in 2000), and add the mixture of water and methanol (3:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and warfarin 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 of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Loss on drying <*2.41*> Not more than 4.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 25 mg each of Warfarin Potassium and Warfarin Potassium RS, previously dried, and separately dissolve in the mixture of water and methanol (3:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mixture of water and methanol (3:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of warfarin.

$$\text{Amount (mg) of } C_{19}H_{15}KO_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Warfarin Potassium RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized 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, acetonitrile and acetic acid (100) (68:32:1).

Flow rate: Adjust the flow rate so that the retention time of warfarin 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 the symmetry factor of the peak of warfarin are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Warfarin Potassium Tablets

ワルファリンカリウム錠

Warfarin Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$: 346.42).

Method of preparation Prepare as directed under Tablets, with Warfarin Potassium.

Identification **(1)** Determine the absorption spectrum of the solution T_2 obtained in the Assay, using 0.02 mol/L potassium hydroxide TS as the blank, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 306 nm and 310 nm, and a minimum between 258 nm and 262 nm. Separately, determine the absorption spectrum of the solution T_1 obtained in the Assay, using 0.02 mol/L hydrochloric acid TS as the blank, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 281 nm and 285 nm and between 303 nm and 307 nm, and a minimum between 243 nm and 247 nm.

(2) Weigh a quantity of Warfarin Potassium Tablets, equivalent to 0.01 g of Warfarin Potassium according to the labeled amount, add 10 mL of acetone, shake, and filter. Heat the filtrate on a water bath to evaporate the acetone. To the residue add 10 mL of diethyl ether and 2 mL of dilute hydrochloric acid, and shake: the aqueous layer responds to the Qualitative Tests ⟨*1.09*⟩ (1) for potassium salt.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Powder 1 tablet of Warfarin Potassium Tablets, add 40 mL of water, and shake vigorouly for 30 minutes. Add water to make exactly V mL of this solution containing about 20 μg of warfarin potassium ($C_{19}H_{15}KO_4$) per ml. Filter this solution, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of Warfarin Potassium 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 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and use these solutions as the solution T_1 and the solution S_1, respectively. Separately, pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L potassium hydroxide TS to make exactly 25 mL, and use these solutions as the solution T_2 and the solution S_2, respectively. Determine the absorbances, A_T and A_S, of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the solution T_2 and the solution S_2 as the blank, respectively.

Amount (mg) of warfarin potassium ($C_{19}H_{15}KO_4$)
$= M_S \times A_T/A_S \times V/2000$

M_S: Amount (mg) of Warfarin Potassium RS

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 0.5-mg, 1-mg and 2-mg tablet and in 30 minutes of 5-mg tablet of Warfarin Potassium Tablets are not less than 80%.

Start the test with 1 tablet of Warfarin Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of warfarin potassium ($C_{19}H_{15}KO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Warfarin Potassium RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add 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. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of warfarin from each solution.

Dissolution rate (%) with respect to the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$

M_S: Amount (mg) of Warfarin Potassium RS
C: Labeled amount (mg) of warfarin potassium ($C_{19}H_{15}KO_4$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside 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°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of warfarin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of warfarin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Warfarin Potassium Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of warfarin potassium ($C_{19}H_{15}KO_4$), add 80 mL of water, shake vigorously for 15 minutes, and add water to make exactly 100 mL. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 80 mg of Warfarin Potassium 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 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, add 0.02 mol/L hydrochloric acid TS to

make exactly 20 mL, and use these solutions as the solution T_1 and the solution S_1, respectively. Separately, pipet 10 mL each of the sample solution and standard solution, add 0.02 mol/L potassium hydroxide TS to make exactly 20 mL, and use these solutions as the solution T_2 and the solution S_2, respectively. Determine the absorbances, A_T and A_S, of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the solution T_2 and the solution S_2 as the blank, respectively.

$$\text{Amount (mg) of warfarin potassium } (C_{19}H_{15}KO_4) = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of Warfarin Potassium RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Water

常水

H_2O: 18.02

Water must meet the Quality Standards of Drinking water provided under the Article 4 of the Water Supply Law (the Ministry of Health, Labour and Welfare Ministerial Ordinance No.101, May 30, 2003). In the case that Water is prepared at individual facilities using well water or industrial water as source water, it must meet the following additional requirement as well as the Quality Standards of Drinking water:

Purity Ammonium <*1.02*>—Perform the test with 30 mL of Water as directed under Ammonium Limit Test. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

Water for Injection

注射用水

Water for Injection is prepared by distillation or by reverse osmosis and/or ultrafiltration, either from Water after applying appropriate pretreatments such as ion-exchange or reverse osmosis, or from Purified Water.

When Water for Injection is prepared by the reverse osmosis and/or ultrafiltration (methods for refining water by using a reverse osmosis membrane module, an ultrafiltration membrane module capable of removing substances having molecular masses of 6,000 and above, or a module using both types of membranes), care must be taken to avoid microbial contamination of the water processing system, and to provide water with equivalent quality to that prepared by distillation consistently.

Water for Injection must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature, are established.

Description Water for Injection is a clear and colorless liquid, having no odor.

Purity Total organic carbon <*2.59*>—Not more than 0.50 mg/L.

Conductivity <*2.51*> When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 $\mu S \cdot cm^{-1}$.

Transfer a suitable amount of Water for Injection to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Bacterial endotoxins <*4.01*> Less than 0.25 EU/mL.

Purified Water

精製水

Purified Water is prepared from Water by ion-exchange, distillation, reverse osmosis or ultrafiltration, or by a combination of these processes.

It must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures for preventing microbial proliferation are taken.

Description Purified Water is a clear and colorless liquid, having no odor.

Purity Total organic carbon <*2.59*>—Not more than 0.50 mg/L.

Conductivity <*2.51*> When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 $\mu S \cdot cm^{-1}$.

Transfer a suitable amount of Purified Water to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Purified Water in Containers

精製水(容器入り)

Purified Water in Containers is prepared from Purified Water by introducing it in a tight container.

It is allowable to describe it as ''Purified Water'' on the label.

Description Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Purified Water in Containers add 10 mL of dilute

sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity ⟨*2.51*⟩ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 $\mu S \cdot cm^{-1}$ for containers with a nominal volume of 10 mL or less, and not more than 5 $\mu S \cdot cm^{-1}$ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Purified Water in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Microbial limit ⟨*4.05*⟩ The acceptance criteria of TAMC is 10^2 CFU/mL. Perform the test using soybean-casein digest agar medium.

Containers and storage Containers—Tight containers.

Sterile Purified Water in Containers

滅菌精製水(容器入り)

Sterile Purified Water in Containers is prepared from Purified Water by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Description Sterile Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Purified Water in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity ⟨*2.51*⟩ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 $\mu S \cdot cm^{-1}$ for containers with a nominal volume of 10 mL or less, and not more than 5 $\mu S \cdot cm^{-1}$ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Purified Water in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Sterility ⟨*4.06*⟩ It meets the requirements.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections can be used in place of hermetic containers.

Sterile Water for Injection in Containers

注射用水(容器入り)

Sterile Water for Injection in Containers is prepared from Water for Injection by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

It is allowable to describe it as ''Water for Injection'' on the label.

For Sterile Water for Injection in Containers prepared from Water for Injection obtained by distillation, an alternative name of ''Distilled Water for Injection'' may be used.

Description Sterile Water for Injection in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Water for Injection in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity ⟨*2.51*⟩ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 $\mu S \cdot cm^{-1}$ for containers with a nominal volume of 10 mL or less, and not more than 5 $\mu S \cdot cm^{-1}$ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Water for Injection in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirements.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ It meets the requirement.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections can be used in place of hermetic containers.

Weil's Disease and Akiyami Combined Vaccine

ワイル病秋やみ混合ワクチン

Weil's Disease and Akiyami Combined Vaccine is a liquid for injection containing inactivated Weil's disease leptospira, Akiyami A leptospira, Akiyami B leptospira and Akiyami C leptospira.

The product lacking more than a kind of Akiyami leptospira may be prepared, if necessary.

It conforms to the requirements of Weil's Disease and Akiyami Combined Vaccine in the Minimum Requirements for Biological Products.

Description Weil's Disease and Akiyami Combined Vaccine is a white-turbid liquid.

Wheat Starch

Amylum Tritici

コムギデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Wheat Starch consists of the starch granules obtained from caryopsis of wheat, *Triticum aestivum* Linné (*Gramineae*).

◆**Description** Wheat Starch occurs as white masses or powder.

It is practically insoluble in water and in ethanol (99.5).◆

Identification **(1)** Examine under a microscope <*5.01*> using a mixture of water and glycerinol (1:1), Wheat Starch presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually 10 – 60 μm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2 – 10 μm in diameter. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Wheat Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): a deep blue color is formed, and the color disappears by heating.

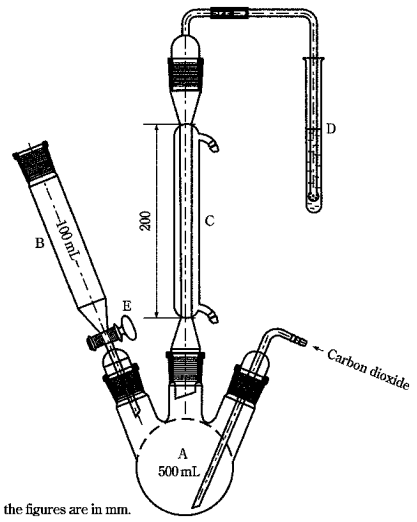
pH <*2.54*> Put 5.0 g of Wheat Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 4.5 and 7.0.

Purity **(1)** Iron—To 1.5 g of Wheat Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (2 in 10) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Wheat Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <*2.50*> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Boiling flask (500 mL)
B: Funnel (100 mL)
C: Condenser
D: Test-tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Wheat Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the

tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

Amount (ppm) of sulfur dioxide = $V/M \times 1000 \times 3.203$

M: Amount (g) of the sample
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

◆**(4)** Foreign matter. Under a microscope <5.01>, Wheat Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any of fragments of the tissue of the original plant.◆

Loss on drying <2.41> Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers.◆

White Ointment

白色軟膏

Method of preparation

White Beeswax	50 g
Sorbitan Sesquioleate	20 g
White Petrolatum	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with the above materials.

Description White Ointment is white in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Whole Human Blood

人全血液

Whole Human Blood is a liquid for injection which is prepared by mixing human blood cells and an anticoagulant solution for storage.

It conforms to the requirements of Whole Human Blood in the Minimum Requirements for Biological Products.

Description Whole Human Blood is a deep red liquid from which the erythrocytes settle upon standing, leaving a yellow supernatant layer. A gray layer which mainly consists of leucocytes may appear on the surface of the settled erythrocyte layer. The supernatant layer may become turbid in the presence of fat, or may show the faint color of hemoglobin.

Wine

ブドウ酒

Wine is an alcoholic liquid obtained by fermenting the juice of the fruits of *Vitis vinifera* Linné (*Vitaceae*) or allied plants.

It contains not less than 11 vol% and not more than 14 vol% of ethanol (C_2H_6O: 46.07) (by specific gravity), and not less than 0.10 w/v% and not more than 0.40 w/v% of L-tartaric acid ($C_4H_6O_6$: 150.09).

It contains no artificial sweetener and no artificial coloring agent.

Description Wine is a light yellow or reddish purple to red-purple liquid. It has a characteristic and aromatic odor. It has a slightly astringent and faintly irritating taste.

Specific gravity <2.56> d_{20}^{20}: 0.990 – 1.010

Optical rotation <2.49> Boil 160 mL of Wine, neutralize with potassium hydroxide TS, and concentrate to 80 mL on a water bath. Cool, dilute with water to 160 mL, add 16 mL of lead subacetate TS, shake well, and filter. To 100 mL of the filtrate add 10 mL of a saturated solution of sodium sulfate decahydrate, shake well, filter, and use the filtrate as the sample solution. Allow 20 mL of the sample solution to stand for 24 hours, add 0.5 g of activated charcoal, shake, stopper, and allow to stand for 10 minutes. Filter, and observe the optical rotation of the filtrate in a 200-mm cell. Multiply the optical rotation observed by 1.21, and designate as the optical rotation of Wine: it is between −0.3° and +0.3°.

Purity **(1)** Total acid [as L-tartaric acid ($C_4H_6O_6$)]—To exactly 10 mL of Wine add 250 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 1 mL of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 7.504 mg of $C_4H_6O_6$

Total acid is not less than 0.40 w/v% and not more than 0.80 w/v%.

(2) Volatile acid [as acetic acid ($C_2H_4O_2$: 60.05)]—Transfer 100 mL of Wine to a beaker, add 1 mL of 1 mol/L sodium hydroxide VS and the same volume of 1 mol/L sodium hydroxide VS as that of 0.1 mol/L sodium hydroxide VS titrated in (1) to make the solution alkaline, and concentrate to 50 mL on a water bath. Cool, add water to make 100 mL, transfer to a 1000-mL distillation flask, containing previously added 100 g of sodium chloride. Wash the beaker with 100 mL of water, and combine the washings in the distillation flask. Add 5 mL of a solution of L-tartaric acid (3 in 20), and distil with steam cautiously to maintain the volume of the solution in the flask until 450 mL of the distillate is obtained for 45 minutes. Dilute the distillate to exactly 500 mL with water, and use this solution as the sample solution. Titrate <2.50> a 250-mL portion of the sample solution with

0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

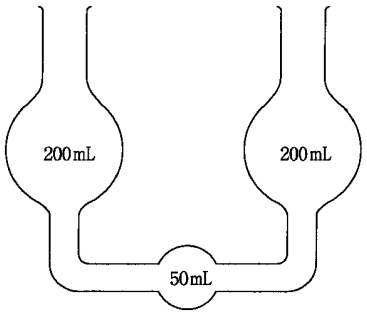
Each mL of 0.1 mol/L sodium hydroxide VS
= 6.005 mg of $C_2H_4O_2$

The volatile acid is not more than 0.15 w/v%.

(3) Sulfur dioxide—Stopper a 750-mL round-bottomed flask with a stopper having two holes. Through one hole, insert a glass tube A extending nearly to the bottom of the flask. Through the other hole, insert a glass tube B ending to the neck of the flask. Connect the tube B to a Liebig's condenser, and the end of the condenser to a joint of which inner diameter is 5 mm at the lower end. Connect the other end of the joint with a holed rubber stopper to a U tube having three bulbs as shown in the Figure. Pass carbon dioxide washed with a solution of potassium permanganate (3 in 100) through the tube A. Displace the air in the apparatus by carbon dioxide, and place 50 mL of a freshly prepared and diluted starch TS (1 in 5) and 1 g of potassium iodide in the U tube. From the other end of the U tube, add 1 to 2 drops of 0.01 mol/L iodine VS from a burette. While passing carbon dioxide, remove the stopper of the flask a little, add exactly 25 mL of Wine, 180 mL of freshly boiled and cooled water, 0.2 g of tannic acid, and 30 mL of phosphoric acid, and stopper again. Pass carbon dioxide for further 15 minutes, heat the distillation flask with caution so that 40 to 50 drops of the distillate may be obtained in 1 minute. When the color of starch TS in the U tube is discharged, add 0.01 mol/L iodine VS dropwise from a burette so that the color of the starch TS remains light blue to blue during the distillation. Read the volume of 0.01 mol/L iodine VS consumed when exactly 60 minutes have passed after the beginning of distillation. In this case, however, the coloration of starch TS produced by 1 drop of 0.01 mol/L iodine VS should persist at least for 1 minute.

Each mL of 0.01 mol/L iodine VS = 0.6406 mg of SO_2

The amount of sulfur dioxide (SO_2: 64.06) does not exceed 7.5 mg.



(4) Total sulfuric acid—Transfer 10 mL of Wine to a beaker, boil, and add 50 mL of a solution prepared by dissolving 5.608 g of barium chloride dihydrate in 50 mL of hydrochloric acid and water to make 1000 mL. Cover the beaker, and heat on a water bath for 2 hours, supplying the water lost by distillation. Cool, centrifuge, and decant the supernatant liquid in another beaker. To this solution add 1 to 2 drops of dilute sulfuric acid, and allow to stand for 1 hour: a white precipitate is formed.

(5) Arsenic <*1.11*>—Evaporate 10 mL of Wine on a water bath to dryness. Prepare the test solution with the residue according to Method 3, and perform the test (not more than 0.2 ppm).

(6) Glycerin—Pipet 100 mL of Wine into a 150-mL porcelain dish, and concentrate on a water bath to 10 mL. Add 1 g of sea sand (No. 1), and make the solution strongly alkaline by adding a solution prepared by dissolving 4 g of calcium hydroxide in 6 mL of water. Heat on a water bath with constant stirring and pushing down any material adhering to the wall of the dish until the contents of the dish become soft masses. Cool, add 5 mL of ethanol (99.5), and grind to a grue-like substance. Heat on a water bath, add 10 to 20 mL of ethanol (99.5) while agitating, boil, and transfer to a 100-mL volumetric flask. Wash the dish with seven 10-mL portions of hot ethanol (99.5), combine the washings with the contents of the flask, cool, and add ethanol (99.5) to make exactly 100 mL. Filter through a dry filter paper, evaporate 90 mL of the filtrate on a water bath, taking care not to boil the solution during the evaporation. Dissolve the residue in a small amount of ethanol (99.5), transfer to a 50-mL glass-stoppered volumetric cylinder, wash with several portions of ethanol (99.5), and add the washings to the solution in the cylinder to make 15 mL. Add three 7.5-mL portions of dehydrated diethyl ether, shake vigorously each time, and allow to stand. When the solution becomes quite clear, transfer to a tared, flat weighing bottle. Wash the volumetric cylinder with 5 mL of a mixture of dehydrated diethyl ether and ethanol (99.5) (3:2). Transfer the washings to the weighing bottle, and evaporate carefully on a water bath. When the liquid becomes sticky, dry at 105°C for 1 hour, and cool in a desiccator (silica gel), and weigh: the mass of the residue is not less than 0.45 g and not more than 0.90 g.

(7) Reducing sugars—To a 25-mL portion of the sample solution obtained in the Optical rotation add 50 mL of boiling Fehling's TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared glass filter by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

(8) Sucrose—Transfer a 50-mL portion of the sample solution obtained in the Optical rotation to a 100-mL flask, neutralize with diluted hydrochloric acid (1 in 30), followed by further addition of 5 mL of diluted hydrochloric acid (1 in 30). Heat in a water bath for 30 minutes, cool, neutralize with a solution of potassium hydroxide (1 in 100), add 4 drops of sodium carbonate TS, filter into a 100-mL volumetric flask, wash with water, combine the washings with the filtrate, and add water to make 100 mL. To 25 mL of this solution add 50 mL of boiling Fehling's TS, and proceed as directed in (7), and weigh as copper (II) oxide. From the number obtained by multiplying the mass (g) of copper (II) oxide by 2, deduct the amount (g) of copper (II) oxide determined in (7), and multiply again the number so obtained by 1.2: the number obtained does not exceed 0.104 (g).

(9) Benzoic acid, cinnamic acid and salicylic acid—Transfer exactly 50 mL of the sample solution obtained in (2) to a separator, add 10 g of sodium chloride and 2 mL of dilute hydrochloric acid, and extract with three 10-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with

two 5-mL portions of water, and extract with three 10-mL portions of 0.1 mol/L sodium hydroxide VS. Combine the alkaline extracts, evaporate the diethyl ether by warming on a water bath, cool, neutralize with 1 mol/L hydrochloric acid VS, and add 5 mL of potassium chloride-hydrochloric acid buffer solution and water to make exactly 50 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24> with this solution, using a solution prepared in the same manner instead of the sample solution as the blank: the absorbance does not exceed 0.15 at a wavelength between 220 nm and 340 nm.

(10) Boric acid—Transfer 50 mL of Wine to a porcelain dish, add 5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite: a half portion of the residue does not respond to Qualitative Tests <1.09> (1) for borate. Dissolve another half portion of the residue in 5 mL of hydrochloric acid: it does not respond to Qualitative Tests <1.09> (2) for borate.

(11) Methanol—Wine meets the requirements of the Methanol Test <1.12>, when proceeding with exactly 1 mL of ethanol layer obtained by Method 1 of the Alcohol Number Determination <1.01> and distilling without adding water after shaking with 0.5 g of calcium carbonate.

(12) Formaldehyde—To 25 mL of Wine add 5 g of sodium chloride and 0.2 g of L-tartaric acid, distil, and obtain 15 mL of the distillate. To 5 mL of the distillate add 5 mL of acetyl acetone TS, mix, and heat on a water bath for 10 minutes: the solution has no more color than that of the following control solution.

Control solution: Using 5 mL of water instead of the distillate, perform the test in the same manner.

Extract content 1.9 – 3.5 w/v% Pipet 25 mL of Wine to a 200-mL tared beaker containing 10 g of sea sand (No. 1), previously dried at 105°C for 2.5 hours, and evaporate to dryness on a water bath. Dry the residue at 105°C for 2 hours, cool in a desiccator (silica gel), and weigh.

Total ash 0.13 – 0.40 w/v% Pipet 50 mL of Wine to a tared porcelain dish, and evaporate to dryness on a water bath. Ignite the residue to the constant mass, cool, and weigh.

Assay (1) Ethanol—Pipet Wine into a 100-mL volumetric flask at 15°C, transfer to a 300- to 500-mL flask, and wash this volumetric flask with two 15-mL portions of water. Add the washings to the sample in the flask, connect the flask to a distillation tube having a trap, and distil using the volumetric flask as a receiver. When about 80 mL of the distillate is obtained (it takes about 20 minutes), stop the distillation, allow to stand in water at 15°C for 30 minutes, and add water to make exactly 100 mL. Shake well, and determine the specific gravity at 15°C according to Method 3 under Specific Gravity <2.56>: the specific gravity d_{15}^{15} is between 0.982 and 0.985.

(2) L-Tartaric acid—Pipet 100 mL of Wine, add 2 mL of acetic acid (100), 0.5 mL of a solution of potassium acetate (1 in 5) and 15 g of powdered potassium chloride, and shake vigorously to dissolve as much as possible. Add 10 mL of ethanol (95), rub the inner wall of the beaker strongly for 1 minute to induce the crystallization, and allow to stand between 0°C and 5°C for more than 15 hours. Filter the crystals by suction, wash successively the beaker and the crystals with 3-mL portions of a solution prepared by dissolving 15 g of powdered potassium chloride in 120 mL of diluted ethanol (1 in 6), and repeat the washings five times. Transfer the crystals together with the filter paper to a beaker, wash the filter with 50 mL of hot water, combine the washings in the beaker, and dissolve the crystals by heating. Titrate <2.50> the solution with 0.2 mol/L sodium hydroxide VS immediately (indicator: 1 mL of phenolphthalein TS). The number obtained by adding 0.75 to the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed represents the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed.

Each mL of 0.2 mol/L sodium hydroxide VS
= 30.02 mg of $C_4H_6O_6$

Containers and storage Containers—Tight containers.

Wood Creosote

木クレオソート

Wood Creosote is a mixture of phenols obtained from by using wood tar derived from dry distillation of stems and branches of various plants of genus *Pinus* (*Pinaceae*), genus *Cryptomeria* (*Taxodiaceae*), genus *Fagus* (*Fagaceae*), genus *Afzelia* (genus *Intsia*); (*Leguminosae*), genus *Shorea* (*Dipterocarpaceae*) or genus *Tectona* (*Verbenaceae*), followed by distillation and collection at 180 to 230°C, then further purification and then re-distillation.

Wood Creosote contains not less than 23.0% and not more than 35% of guaiacol ($C_7H_8O_2$: 124.14).

Description Wood Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor.

It is slightly soluble in water.

It is miscible with methanol and with ethanol (99.5).

Its saturated solution is acidic.

It is highly refractive.

It gradually changes in color by light or by air.

Identification Use the sample solution obtained in the Assay as the sample solution. Separately, dissolve 0.1 g of phenol, *p*-cresol, guaiacol, and 2-methoxy-4-methylphenol in methanol respectively, to make 100 mL. To 10 mL of each solution add methanol to make 50 mL, and use these solutions as standard solution (1), standard solution (2), standard solution (3) and standard solution (4). Perform the test with 10 μL each of the sample solution, standard solution (1), (2), (3) and (4) as directed under Liquid Chromatography <2.01> according to the following conditions: the main peaks obtained with the sample solution show the same retention times with those obtained with the standard solutions (1), (2), (3) and (4).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

Specific gravity <2.56> d_{20}^{20}: not less than 1.076.

Purity (1) Coal Creosote—Accurately measure 10 mL of Wood Creosote, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately to 1 mg each of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene add a small quantity of ethyl acetate,

if necessary, and add methanol to make 100 mL. To 1 mL of this solution add methanol to make 100 mL, and use this soiution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions: No peaks are detected with the sample solution for the retention times corresponding to benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene of the standard solution. Change these conditions if any peak is detected for retention times that correspond to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*,*h*]anthracene, to verify that such a peak does not belong to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*,*h*]anthracene.

Operating conditions—

Detector: A mass spectrometer (EI).

Monitored ions:

Benz[*a*]anthracene: Molecular ion *m/z* 228, Fragment ion *m/z* 114	About 14 to 20 minutes
Benzo[*a*]pyrene: Molecular ion *m/z* 252, Fragment ion *m/z* 125	About 20 to 25 minutes
Dibenz[*a*,*h*]anthracene: Molecular ion *m/z* 278, Fragment ion *m/z* 139	About 25 to 30 minutes

Column: A quartz tube 0.25 mm in inside diameter and 30 m in length, with internal coating 0.25 – 0.5 μm in thickness made of 5% diphenyl-95% dimethyl polysiloxane for gas chromatography.

Column temperature: Inject sample at a constant temperature in vicinity of 45°C, then raise temperature to 240°C at the rate of 40°C per minute, maintain the temperature at 240°C for 5 minutes, then raise temperature to 300°C at the rate of 4°C per minute, then raise the temperature to 320°C at the rate of 10°C per minute, then maintain temperature at 320°C for 3 minutes.

Injection port temperature: A constant temperature in vicinity of 250°C.

Interface temperature: A constant temperature in vicinity of 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo[*a*]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of standard solution and add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the test is performed with conditions described above with 1 μL of the solution for system suitability test, the SN ratio of each substance is not less than 3.

System performance: When the procedure is run with conditions described above with 1 μL of the solution for system suitability test, the elution takes place in order of benz[*a*]anthracene, benzo[*a*]pyrene and then dibenz[*a*,*h*]anthracene.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene is respectively not more than 10%.

(2) Acenaphthene—To 0.12 g of Wood Creosote add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of acenaphthene in methanol to make 50 mL. To 5 mL of this solution add methanol to make 20 mL. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions: No peaks are detected with sample solution for the retention time corresponding to acenaphthene of the standard solution. Change these conditions if any peak is detected for the retention time corresponding to acenaphthene, to verify that such a peak does not belong to athenaphthene.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.25 mm inside diameter and 60 m in length, with internal coating 0.25. 0.5 μm in thickness made of polymethylsiloxane for gas chromatography.

Column temperature: Perform injection at a constant temperature in vicinity of 45°C, then raise the temperature by 11.5°C per minute until reaching 160°C, then raise the temperature by 4°C per minute until reaching 180°C, then raise the temperature by 8°C until reaching 270°C, then maintain temperature at 270°C for 3 minutes.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acenaphthene is about 18 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of the standard solution, add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the procedure is run with conditions described above with 1 μL of solution for system suitability test, the SN ratio of acenaphthene is not less than 3.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acenaphthene is not more than 6.0%.

(3) Other impurities

Add 2 mL of petroleum benzin to 1.0 mL of Wood Creosote, then add 2 mL of barium hydroxide test solution, agitate to mix and allow to stand. No blue or muddy brown color develops in the upper layer of the mixture. Furthermore, no red color develops in the lower layer.

Distilling range <2.57> 200 – 220°C, not less than 85 vol%.

Assay To about 0.1 g of Wood Creosote, accurately weighed, add methanol to make exactly 50 mL. Pipet 10 mL of this solution add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, add methanol to about 30 mg of accurately measured guaiacol for assay to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of guaiacol for each solution.

$$\text{Amount (mg) of guaiacol } (C_7H_8O_2) = M_S \times A_T/A_S$$

M_S: Amount of guaiacol for assay (mg)

Operating conditions—

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

Column: Fill a stainless steel tube with internal diameter of 4.6 mm and length of 15 cm with 5 μm of octadecylsilanized silica gel for gas chromatography.

Column temperature: A constant temperature in vicinity of 40°C.

Mobile phase: Mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of guaiacol is about 9 minutes.

System suitability—

System performance: Dissolve 2 mg each of guaiacol and phenol in methanol to make 10 mL. The procedure is run with conditions described above with 10 μL of this solution, the elution takes place in order of phenol then guaiacol, with the degree in separation of not less than 2.5.

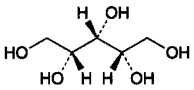
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Xylitol

キシリトール



$C_5H_{12}O_5$: 152.15

meso-Xylitol

[*87-99-0*]

Xylitol, when dried, contains not less than 98.0% of $C_5H_{12}O_5$.

Description Xylitol occurs as white crystals or powder. It is odorless and has a sweet taste.

It is very soluble in water, slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 1 mL of a solution of Xylitol (1 in 2) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): blue-green color is produced without turbidity.

(2) Determine the infrared absorption spectrum of Xylitol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 5.0 g of Xylitol in 10 mL of freshly boiled and cooled water: the pH of this solution is between 5.0 and 7.0.

Melting point <*2.60*> 93.0 – 95.0°C

Purity **(1)** Clarity and color of solution—Dissolve 5 g of Xylitol in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 2.0 g of Xylitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <*1.14*>—Perform the test with 4.0 g of Xylitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <*1.07*>—Proceed with 4.0 g of Xylitol according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Dissolve 0.5 g of Xylitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color is produced.

(6) Arsenic <*1.11*>—Prepare the test solution with 1.5 g of Xylitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Sugars—Dissolve 5.0 g of Xylitol in 15 mL of water, add 4.0 mL of dilute hydrochloric acid, and heat in a water bath for 3 hours under a reflux condenser. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS). Then add water to make 50 mL, transfer 10 mL of this solution to a flask, add 10 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand to precipitate copper (I) oxide. Remove the supernatant liquid through a glass filter (G4), and wash the precipitate with warm water until the last washing does not show alkalinity. Filter these washings through the glass filter mentioned above. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter the solution through the glass filter mentioned above, wash with water, combine the washings with the filtrate, heat at 80°C, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS: not more than 1.0 mL of 0.02 mol/L potassium permanganate VS is consumed.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Xylitol, previously dried, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into an iodine flask, add 50 mL of potassium periodate TS exactly, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, stopper, shake well, allow to stand for 5 minutes in a dark place, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

$$\text{Each mL of 0.1 mol/L sodium thiosulfate VS} = 1.902 \text{ mg of } C_5H_{12}O_5$$

Containers and storage Containers—Tight containers.

Xylitol Injection

キシリトール注射液

Xylitol Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of xylitol ($C_5H_{12}O_5$: 152.15).

Method of preparation Prepare as directed under Injections, with Xylitol.

No preservative may be added.

Description Xylitol Injection is a clear, colorless liquid. It has a sweet taste.

Identification Measure a volume of Xylitol Injection, equivalent to 0.1 g of Xylitol according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.1 g of xylitol in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), ammonia solution (28) and water (25:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly silver nitrate-ammonia TS, and dry at 105°C for 15 minutes: the spots from the sample solution and standard solution show a blackish brown color and the same *R*f value.

pH <*2.54*> 4.5 – 7.5

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Xylitol Injection, equivalent to about 5 g of xylitol ($C_5H_{12}O_5$) according to the labeled amount, and add water to make exactly 250 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Then, pipet 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under Xylitol.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.902 mg of $C_5H_{12}O_5$

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.

It contains not less than 400 mg of protein and not less than 100 μg of thiamine compounds [as thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27)] in each 1 g.

Description Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope <*5.01*>, shows isolated cells, spheroidal or oval in shape, and 6 to 12 μm in length.

Purity **(1)** Rancidity—Dried Yeast is free from any unpleasant or rancid odor or taste.

(2) Starch—Add iodine TS to Dried Yeast, and examine microscopically <*5.01*>: no or only a few granules are tinted blackish purple.

Loss on drying <*2.41*> Not more than 8.0% (1 g, 100°C, 8 hours).

Total ash <*5.01*> Not more than 9.0% (1 g).

Assay **(1)** Protein—Weigh accurately about 50 mg of Dried Yeast and perform the test as directed under Nitrogen Determination <*1.08*>.

Amount (mg) of protein in 1 g of Dried Yeast
$= N \times 6.25 \times 1/M$

N: Amount (mg) of nitrogen (N)
M: Amount (g) of sample

(2) Thiamine—Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water bath at 80°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45°C to 50°C for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 to 110 μm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10-mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5-mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this solution as the sample solution. Separately, weigh accurately about 15 mg of Thiamine Chloride Hydrochloride RS (previously determine the water <*2.48*> in the same manner as Thiamine Chloride Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100

mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

$$\text{Amount } (\mu g) \text{ of thiamine in 1 g of Dried Yeast} = M_S/M_T \times Q_T/Q_S \times 12.5$$

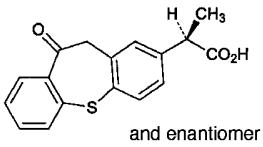
M_S: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis
M_T: Amount (g) of the sample

Internal standard solution—Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 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°C.
Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-octanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.
Selection of column: Proceed with 200 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Zaltoprofen

ザルトプロフェン



and enantiomer

$C_{17}H_{14}O_3S$: 298.36
(2*RS*)-2-(10-Oxo-10,11-dihydrodibenzo[*b*,*f*]thiepin-2-yl)propanoic acid
[*74711-43-6*]

Zaltoprofen, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{14}O_3S$.

Description Zaltoprofen occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed by light.

A solution of Zaltoprofen in acetone (1 in 10) shows no optical rotation.

Identification (1) To 0.2 g of Zaltoprofen add 0.5 g of sodium hydroxide, heat gradually to melt, and then carbonize. After cooling, add 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moisten lead (II) acetate paper.

(2) Determine the absorption spectrum of a solution of Zaltoprofen in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zaltoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 135 – 139°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Zaltoprofen 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 <1.11>—Prepare the test solution with 1.0 g of Zaltoprofen according to Method 3, using 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (2 in 25), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Zaltoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 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 sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than zaltoprofen and the peak having the relative retention time of about 0.7 with respect to zaltoprofen from the sample solution is not larger than the peak area of zaltoprofen from the standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside 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 acetonitrile, water and acetic acid (100) (300:200:1).
Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.
Time span of measurement: About 15 times as long as the retention time of zaltoprofen beginning after the solvent peak.
System suitability—
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 zaltoprofen obtained with 20 μL of this solution is equivalent to 8 to 12% of that

with 20 μL of the standard solution.

System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate in 100 mL of ethanol (99.5). Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, zaltoprofen and isopropyl benzoate 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zaltoprofen is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Zaltoprofen, dissolve in 50 mL of methanol, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.84 mg of $C_{17}H_{14}O_3S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Zaltoprofen Tablets

ザルトプロフェン錠

Zaltoprofen Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zaltoprofen ($C_{17}H_{14}O_3S$: 298.36).

Method of preparation Prepare as directed under Tablets, with Zaltoprofen.

Identification Powder a suitable amount of Zaltoprofen Tablets. To a portion of the powder, equivalent to 80 mg of Zaltoprofen, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the supernatant liquid add ethanol (99.5) to make 20 mL. To 2 mL of this solution add ethanol (99.5) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 227 nm and 231 nm and between 329 nm and 333 nm, and a shoulder between 241 nm and 245 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Zaltoprofen Tablets add 4 mL of water, and shake to disintegrate. Add a suitable amount of ethanol (95), shake, then add ethanol (95) to make exactly V mL so that each mL contains about 4 mg of zaltoprofen ($C_{17}H_{14}O_3S$), and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of zaltoprofen ($C_{17}H_{14}O_3S$)
$= M_S \times Q_T/Q_S \times V/20$

M_S: Amount (mg) of zaltoprofen for assay

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Zaltoprofen Tablets is not less than 75%.

Start the test with 1 tablet of Zaltoprofen Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 44 μg of zaltoprofen ($C_{17}H_{14}O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of ethanol (99.5), and add the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 340 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of zaltoprofen ($C_{17}H_{14}O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of zaltoprofen for assay
C: Labeled amount (mg) of zaltoprofen for assay in 1 tablet

Assay To 10 tablets of Zaltoprofen Tablets add 40 mL of water, shake to disintegrate, then add a suitable amount of ethanol (95), shake, add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 8 mg of zaltoprofen ($C_{17}H_{14}O_3S$), add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, add 4 mL of water and ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of zaltoprofen to that of the internal standard.

Amount (mg) of zaltoprofen ($C_{17}H_{14}O_3S$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of zaltoprofen for assay

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diame-

ter 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 acetonitrile, water and acetic acid (100) (300:200:1).

Flow rate: Adjust the flow rate so that the retention time of zaltoprofen 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, zaltoprofen 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of zaltoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Zidovudine

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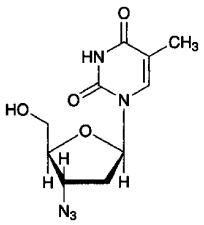


$C_{10}H_{13}N_5O_4$: 267.24
3′-Azido-3′-deoxythymidine
[*30516-87-1*]

Zidovudine contains not less than 97.0% and not more than 102.0% of $C_{10}H_{13}N_5O_4$, calculated on the anhydrous basis.

Description Zidovudine occurs as a white to pale yellowish white powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and sparingly soluble in water.

It gradually turns yellow-brown on exposure to light.

Melting point: about 124°C.

Identification Determine the infrared absorption spectrum of Zidovudine as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Zidovudine RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the RS separately in a small amount of water and dry them in a desiccator (in vacuum, phosphorus (V) oxide), and perform the test with the residues.

Optical rotation <*2.49*> $[\alpha]_D^{25}$: +60.5 – +63.0° (0.5 g calculated on the anhydrous basis, ethanol (99.5), 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>— Proceed with 1.0 g of Zidovudine 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) 1-[(2*R*,5*S*)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl]thymine, triphenylmethanol, and other related substances—Dissolve 0.20 g of Zidovudine in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, add 1 mL of the sample solution to 20 mg each of thymine for liquid chromatography, 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatography, and triphenylmethanol for thin-layer chromatography, and add methanol to dissolve to make exactly 100 mL. Pipet 5 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 <*2.03*>. Spot 10 μL each of the sample 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 and methanol (9:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution that corresponds to the position of the 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine obtained from the standard solution is not more intense than the spot from the standard solution, and the spot other than the principal spot and spots other than thymine and 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine from the sample solution is not more intense than zidovudine spot from the standard solution. However, the 3 spots from the standard solution appear in ascending order of *R*f value thymine, 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine, and zidovudine. Furthermore, spray evenly on the plate a solution of vanillin in sulfuric acid (1 in 100): the spot from the sample solution corresponding to the spot of triphenylmethanol from the standard solution is not more intense than the spot from the standard solution.

(3) Thymine, 3′-chloro-3′-deoxythymidine, and other related substances—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 20 mg of thymine for liquid chromatography, dissolve in 100 mL of methanol, and add the mobile phase to make exactly 250 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the thymine peak areas, A_T and A_S, of the sample and standard solutions, and calculate the amount of thymine using the following formula: the amount is not more than 2.0%. Also, determine the peak area of each peak obtained from the sample solution by the automatic integration method, and calculate the amounts of related substances other than thymine by the area percentage method: the amount of 3′-chloro-3′-deoxythymidine, whose relative retention time to zidovudine is 1.2, is not more than 1.0%, and is not more than 0.5% for all other related substances. Finally, the total amount of thymine, 3′-chloro-3′-deoxythymidine, and all related substances obtained above is not more than 3.0%.

$$\text{Amount (\%) of thymine} = M_S/M_T \times A_T/A_S \times 10$$

M_S: Amount (mg) of thymine for liquid chromatography
M_T: Amount (mg) of Zidovudine

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time of zidovudine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of zidovudine obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that from 10 μL of the solution for system suitability test.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <*2.48*> Not more than 1.0% (0.25 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 50 mg of Zidovudine and Zidovudine RS (separately determine the water <*2.48*> using the same manner as Zidovudine), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of each solution, add the mobile phase to make them exactly 50 mL, and use these solutions as the sample and the standard solutions, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas of zidovudine, A_T and A_S of both solutions.

$$\text{Amount (mg) of } C_{10}H_{13}N_5O_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Zidovudine RS, calculated on the anhydrous basis

Operating conditions—

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

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

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (4:1).

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

System suitability—

System performance: Dissolve 50 mg of Zidovudine in 50 mL of the mobile phase. Separately, dissolve 5 mg of 3′-chloro-3′-deoxythymidine for liquid chromatography in 50 mL of the mobile phase. Mix 10 mL and 1 mL of these solutions, respectively, and add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above conditions, zidovudine and 3′-chloro-3′-deoxythymidine are eluted in this order with the resolution between these peaks being not less than 1.4, and the symmetry factor of the peak of zidovudine is not more than 1.5.

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

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Zinc Chloride

塩化亜鉛

$ZnCl_2$: 136.29

Zinc Chloride contains not less than 97.0% of $ZnCl_2$.

Description Zinc Chloride occurs as white, crystalline powder, rods, or masses. It is odorless.

It is very soluble in water, and freely soluble in ethanol (95), and its solution may sometimes be slightly turbid. The solution becomes clear on addition of a small amount of hydrochloric acid.

The pH of an aqueous solution of Zinc Chloride (1 in 2) is between 3.3 and 5.3.

It is deliquescent.

Identification An aqueous solution of Zinc Chloride (1 in 30) responds to the Qualitative Tests <*1.09*> for zinc salt and chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Zinc Chloride in 10 mL of water and 2 drops of hydrochloric acid: the solution has no color, and is clear.

(2) Sulfate <*1.14*>—Perform the test with 2.0 g of Zinc Chloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Ammonium—Dissolve 0.5 g of Zinc Chloride in 5 mL of water, and warm with 10 mL of a solution of sodium hydroxide (1 in 6): the evolving gas does not change moistened red litmus paper to blue.

(4) Heavy metals—Dissolve 0.5 g of Zinc Chloride in 5 mL of water in a Nessler tube, shake thoroughly with 15 mL of potassium cyanide TS, add 1 drop of sodium sulfide TS, allow to stand for 5 minutes, and immediately observe from the top downward against a white background: the solution has no more color than the following control solution.

Control solution: To 2.5 mL of Standard Lead Solution add 3 mL of water and 15 mL of potassium cyanide TS, shake thoroughly, and add 1 drop of sodium sulfide TS (not more than 50 ppm).

(5) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Chloride in 120 mL of water, add ammonium sulfide TS to complete precipitation, add water to make 200 mL, shake thoroughly, and filter through dry filter paper. Discard the first 20 mL of the filtrate, take the following 100 mL of the filtrate, evaporate with 3 drops of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass is not more than 10.0 mg.

(6) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Zinc Chloride according to Method 1, and perform the test (not more than 5 ppm).

(7) Oxychloride—Shake gently 0.25 g of Zinc Chloride

with 5 mL of water and 5 mL of ethanol (95), and add 0.3 mL of 1 mol/L hydrochloric acid VS: the solution is clear.

Assay Weigh accurately about 0.3 g of Zinc Chloride, add 0.4 mL of dilute hydrochloric acid and water to make exactly 200 mL. Measure exactly 20 mL of the solution, add 80 mL of water, 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.363 mg of $ZnCl_2$

Containers and storage Containers—Tight containers.

Zinc Oxide

酸化亜鉛

ZnO: 81.38

Zinc Oxide, when ignited, contains not less than 99.0% of ZnO.

Description Zinc Oxide occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetic acid (100) and in diethyl ether.

It dissolves in diute hydrochloric acid and in sodium hydroxide TS.

It gradually absorbs carbon dioxide from air.

Identification **(1)** Heat Zinc Oxide strongly: a yellow color develops on strong heating, and disappears on cooling.

(2) A solution of Zinc Oxide in dilute hydrochloric acid (1 in 10) responds to the Qualitative Tests <1.09> for zinc salt.

Purity **(1)** Carbonate, and clarity and color of solution—Mix 2.0 g of Zinc Oxide with 10 mL of water, add 30 mL of dilute sulfuric acid, and heat on a water bath with stirring: no effervescence occurs, and the solution obtained is clear and colorless.

(2) Alkalinity—To 1.0 g of Zinc Oxide add 10 mL of water, and boil for 2 minutes. Cool, filter through a glass filter (G3), and to the filtrate add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Sulfate <1.14>—Shake 0.5 g of Zinc Oxide with 40 mL of water, and filter. Take 20 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 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Iron—Dissolve 1.0 g of Zinc Oxide in 50 mL of diluted hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxodisulfate in this solution, and extract with 20 mL of 4-methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4-methyl-2-pentanone layer, extract again, and use the layer of the buffer solution as the test solution. Separately, perform the test in the same manner with 1.0 mL of Standard Iron Solution, and use the layer so obtained as the control solution. Add 2 mL each of L-ascorbic acid solution for Iron Limit Test (1 in 100) to the test solution and the control solution, respectively, mix, allow to stand for 30 minutes, add 5 mL of an ethanol (95) solution of α,α'-dipyridyl (1 in 200) and water to make 50 mL. After allowing to stand for 30 minutes, compare the color of the both liquids against a white back: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) Lead—To 2.0 g of Zinc Oxide add 20 mL of water, then add 5 mL of acetic acid (100) with stirring, and heat on a water bath until solution is complete. Cool, and add 5 drops of potassium chromate TS: no turbidity is produced.

(6) Arsenic <1.11>—Dissolve 0.5 g of Zinc Oxide in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 4 ppm).

Loss on ignitiono20 <2.43> Not more than 1.0% (1 g, 850°C, 1 hour).

Assay Weigh accurately about 0.8 g of Zinc Oxide, previously ignited at 850°C for 1 hour, dissolve in 2 mL of water and 3 mL of hydrochloric acid, and add water to exactly 100 mL. Pipet 10 mL of this solution, add 80 mL of water, then add a solution of sodium hydroxide (1 in 50) until a slight precipitate is produced. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Oil

チンク油

Zinc Oxide Oil contains not less than 45.0% and not more than 55.0% of zinc oxide (ZnO: 81.38).

Method of preparation

Zinc Oxide	500 g
Fixed oil	a sufficient quantity
To make	1000 g

Mix the above ingredients. An appropriate quantity of Castor Oil or polysorbate 20 may be used partially in place of fixed oil.

Description Zinc Oxide Oil is a white to whitish, slimy substance, separating a part of its ingredients when stored for a prolonged period.

Identification Mix thoroughly, and place 0.5 g of Zinc Oxide Oil in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add

2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Assay Weigh accurately about 0.8 g of Zinc Oxide Oil, mixed well, place in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Ointment

亜鉛華軟膏

Zinc Oxide Ointment contains not less than 18.5% and not more than 21.5% of zinc oxide (ZnO: 81.38).

Method of preparation

Zinc Oxide	200 g
Liquid Paraffin	30 g
White Ointment	a sufficient quantity
To make	1000 g

Prepare as directed under Ointments, with the above ingredients. White Beeswax, Sorbitan Sesquioleate or White Petrolatum may be used instead of White Ointment.

Description Zinc Oxide Ointment is white in color.

Identification Place 1 g of Zinc Oxide Ointment in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Purity Calcium, magnesium and other foreign inorganic matters—Place 2.0 g of Zinc Oxide Ointment in a crucible, melt by warming, and heat gradually raising the temperature, until the mass is thoroughly charred. Ignite the mass strongly until the residue becomes uniformly yellow, and cool. Add 6 mL of dilute hydrochloric acid, and heat on a water bath for 5 to 10 minutes: the solution is colorless and clear. Filter the solution, add 10 mL of water to the filtrate, and add ammonia TS until the precipitate first formed redissolves. Add 2 mL each of ammonium oxalate TS and disodium hydrogenphosphate TS to this solution: the solution remains unchanged or becomes very slightly turbid within 5 minutes.

Assay Weigh accurately about 2 g of Zinc Oxide Ointment, place in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes uniformly yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Add 80 mL of water to exactly 20 mL of this solution, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Starch Powder

亜鉛華デンプン

Method of preparation

Zinc Oxide	500 g
Starch	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Zinc Oxide Starch Powder occurs as a white powder.

Identification **(1)** Place 1 g of Zinc Oxide Starch Powder in a crucible, heat gradually, raising the temperature until it is charred, and then ignite strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(2) Shake well 1 g of Oxide Starch Powder with 10 mL of water and 5 mL of dilute hydrochloric acid, and filter. Boil the residue on a filter paper with 10 mL of water, cool, and add 1 drop of iodine TS: a dark blue-purple color is produced (starch).

Containers and storage Containers—Tight containers.

Zinc Sulfate Hydrate

硫酸亜鉛水和物

$ZnSO_4.7H_2O$: 287.55

Zinc Sulfate Hydrate contains not less than 99.0% and not more than 102.0% of $ZnSO_4.7H_2O$.

Description Zinc Sulfate Hydrate occurs as colorless crystals or white crystalline powder.

It is very soluble in water, and very slightly soluble in

ethanol (99.5).

It effloresces in dry air.

Identification **(1)** A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for zinc salt.

(2) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 1.0 g of Zinc Sulfate Hydrate in 20 mL of water: the pH of the solution is between 4.4 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g of Zinc Sulfate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Dissolve 1.0 g of Zinc Sulfate Hydrate in 10 mL of water contained in a Nessler tube. Add 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS, and allow the mixture to stand for 5 minutes. Observe vertically against a white background, the color of the solution is not more intense than the following control solution.

Control solution: To 1.0 mL of Standard Lead Solution add 10 mL of water and 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS (not more than 10 ppm).

(3) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Sulfate Hydrate in 150 mL of water, add a suitable amount of ammonium sulfide TS to complete the precipitation, and add water to make exactly 200 mL. Shake well, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, take exactly 100 mL of the subsequent filtrate, evaporate to dryness, and ignite as directed under Residue on Ignition <*2.44*>: the mass of the residue is not more than 5.0 mg.

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Zinc Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not less than 35.5% and not more than 38.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.3 g of Zinc Sulfate Hydrate, and dissolve in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.01 mol/L disodium dihydorogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.876 mg of $ZnSO_4.7H_2O$

Containers and storage Containers—Tight containers.

Zinc Sulfate Ophthalmic Solution

硫酸亜鉛点眼液

Zinc Sulfate Ophthalmic Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of zinc sulfate hydrate ($ZnSO_4.7H_2O$: 287.55).

Method of preparation

Zinc Sulfate Hydrate	3 g
Boric Acid	20 g
Sodium Chloride	5 g
Fennel Oil	2 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Ophthalmic Solution, with the above ingredients.

Description Zinc Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification **(1)** Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <*1.09*> for zinc salt.

(2) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <*1.09*> for borate.

(3) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <*1.09*> for chloride.

Assay Pipet accurately 25 mL of Zinc Sulfate Ophthalmic Solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

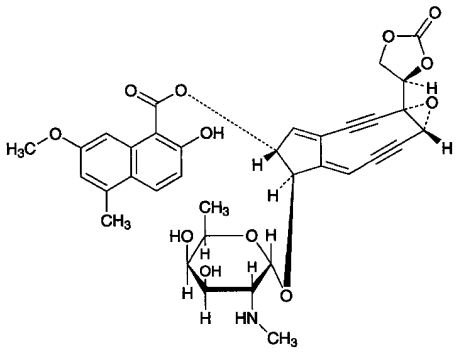
Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.876 mg of $ZnSO_4.7H_2O$

Containers and storage Containers—Tight containers.

Zinostatin Stimalamer

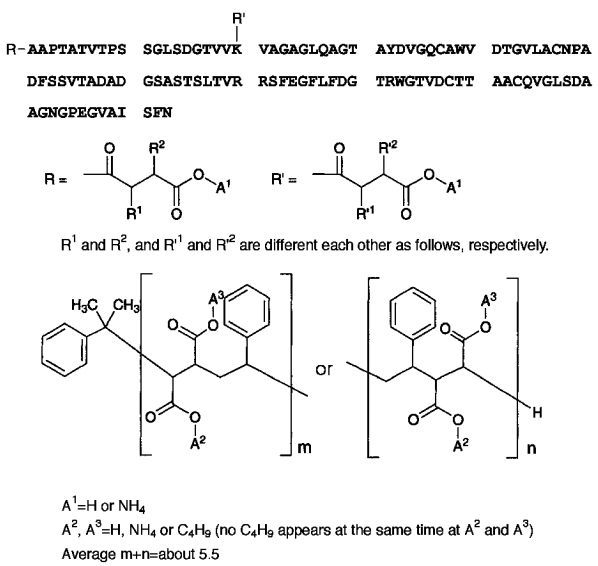
ジノスタチン スチマラマー

Chromophore moiety



(4*S*,6*R*,11*R*,12*R*)-11-[α-D-2,6-Dideoxy-2-(methylamino)-galactopyranosyloxy]-4-[(4*R*)-2-oxo-1,3-dioxolan-4-yl]-5-oxatricyclo[8.3.0.0^{4,6}]trideca-1(13),9-diene-2,7-diyn-12-yl 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylate

Apoprotein moiety bonded to styrene-maleic acid alternate copolymer



A^1=H or NH_4

A^2, A^3=H, NH_4 or C_4H_9 (no C_4H_9 appears at the same time at A^2 and A^3)

Average m+n=about 5.5

[*123760-07-6*]

Zinostatin Stimalamer consists 1 molecule of zinostatin, consisting of chromophore and apoprotein (polypeptide consisting of 113 amino acid residues) and 2 molecules of partially butyl-esterified styrene-maleic acid alternate copolymer, and has average molecular mass of about 15,000. The alternate copolymer is bound an amido bond to α-amino group of alanine of N-terminal and to ε-amino group of lysine 20 of the apoprotein.

It contains not less than 900 μg (potency) and not more than 1080 μg (potency) per mg, calculated on the anhydrous basis. The potency of Zinostatin Stimalamer is expressed as mass (potency) of zinostatin stimalamer.

Description Zinostatin Stimalamer occurs as a pale yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification **(1)** Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of sodium hydroxide TS, and add a drop of copper (II) sulfate TS: a purple color develops.

(2) Dissolve 1 mg of Zinostatin Stimalamer in 1 mL of 0.05 mol/L phosphate buffer solution, pH 7.0, add 0.5 mL of a solution of trichloroacetic acid (1 in 5), and shake: a white precipitate is formed.

(3) Determine the absorption spectra of solutions of Zinostatin Stimalamer and Zinostatin Stimalamer RS in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Zinostatin Stimalamer and Zinostatin Stimalamer RS as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance *<2.24>* $E^{1\%}_{1\,cm}$ (268 nm): 15.5 – 18.5 (4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL).

Optical rotation *<2.49>* $[\alpha]_D^{20}$: −30.0 – −38.0° (20 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 5 mL, 100 mm).

pH *<2.54>* Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 20 mg of Zinostatin Stimalamer in 2 mL of 0.05 mol/L phosphate buffer solution, pH 5.0: the solution is clear, and the absorbance at 400 nm of this solution after addition of 3 mL of 0.05 mol/L phosphate buffer solution, determined as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, is not more than 0.25.

(2) Heavy metals *<1.07>*—Weigh accurately 40 mg of Zinostatin Stimalamer, place in a crucible, carbonize and incinerate according to Method 2, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. After cooling, weigh the residue M_T g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), add 1 mL of water, 85 μL of diluted ammonia TS (1 in 2) and 0.1 mL of dilute acetic acid, and add water so that the mass is M_T + 2.0 g. Adjust the pH of this solution to 3.2 to 3.4 with diluted ammonia TS (1 in 20) or diluted hydrochloric acid (1 in 50), add water so that the mass is M_T + 2.5 g, and use this solution as the test solution. Separately, prepare the blank solution in the same manner without the sample. Separately, take 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid, and evaporate to dryness according to Method 2. After cooling, weigh the residue M_S g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), and proceed in the same manner as directed in the preparation of the test solution. After adjusting the pH of the solution so obtained to 3.2 to 3.4, add 80 μL of Standard Lead Solution, and add water so that the mass is M_S + 2.5 g, and use this solution as the control solution. Add 10 μL each of diluted sodium sulfide TS (1 in 6) to the test solution, the blank solution and the control solution, mix,

and allow to stand for 5 minutes. Determine the absorbances, A_T, A_O and A_S of the test solution, the blank solution and the control solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: $A_T - A_O$ is not larger than $A_S - A_O$ (not more than 20 ppm).

(3) Styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)—

(i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

(iii) Standard solution Weigh accurately about 6 mg of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis, and dissolve in the buffer solution for sample to make exactly 20 mL. Separately, weigh accurately about 6 mg of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), and dissolve in the buffer solution for sample to make exactly 20 mL. Pipet 1 mL each of these solutions, add the buffer solution for sample to make exactly 20 mL, and use this solution as the standard solution.

(iv) Sample solution Weigh accurately about 5 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the buffer solution for sample to make exactly 10 mL.

(v) Procedure Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 μL each of the sample solution and standard solution onto the surface of separate gels, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vi) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix before using 1 volume of this solution and 2 volumes of water. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the back ground of the gel becomes colorless.

(vii) Determination Determine the peak areas, A_{T1}, A_{T2}, A_{S1} and A_{S2}, of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) obtained from the sample solution and the standard solution, based on the absorbance at 600 nm of the gels determined by using a densitometer. Calculate the amounts of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formulae: their amounts are not more than 3.0%, respectively.

Amount (%) of styrene-maleic acid alternating copolymer partial butyl ester

$$= M_{S1}/M_T \times A_{T1}/A_{S1} \times 5/2$$

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)

$$= M_{S2} \times (P_S/100)/M_T \times A_{T2}/A_{S2} \times 5/2$$

M_{S1}: Amount (mg) of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis

M_{S2}: Amount (mg) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), calculated on the anhydrous basis

M_T: Amount (mg) of sample, calculated on the anhydrous basis

P_S: Purity (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)

(4) Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1)—Weigh accurately about 10 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the mobile phase to make exactly 1 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 10 mg of neocarzinostatin (separately determine the water <2.48> in the same manner as Zinostatin Stimalamer), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 0.2 mL each of the sample stock solution and the standard stock solution, add to them exactly 1.5 mL each of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of a solution of sodium 2,4,6-trinitrobenzenesulfonate (1 in 20), allow to stand for 10 minutes at room temperature, then add

exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use these solutions as the sample solution and the standard solution, respectively. Separately, pipet 0.2 mL of the sample stock solution, add 1.5 mL of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of water, allow to stand for 10 minutes at room temperature, then add exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use this solution as the blank solution. Perform the test with exactly 0.25 mL each of the sample solution, the standard solution and the blank solution as directed under Liquid Chromatography *<2.01>* under the following conditions, and determine the peak area, A_T, of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, the peak area, A_S, of trinitrobenzenesulfonic acid derivative of neocarzinostatin obtained from the standard solution, which retention time is almost the same as that of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, and the peak area, A_0, obtained from the blank solution. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) by the following formula: not more than 5.0%.

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1)
$= M_S/M_T \times (A_T - A_0)/A_S \times 2 \times 2.280$

M_S: Amount (mg) of neocarzinostatin calculated on the anhydrous basis
M_T: Amount (mg) of sample, calculated on the anhydrous basis

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: Pre-column is a stainless steel column 7.5 mm in inside diameter and 7.5 cm in length, packed with silica gel for liquid chromatography (10 μm in particle size). Separation column is a stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with silica gel for liquid chromatography (10 μm in particle size), which is coupled to the pre-column.

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.78 g of potassium dihydrogen phosphate and 5.52 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) is about 21 minutes.

System suitability—

System performance: When the procedure is run with 0.25 mL of the standard stock solution under the above operating conditions excepting at 254 mm, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 0.25 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trinitrobenzenesulfonic acid derivative of neocarzinostatin is not more than 10%.

(5) Manufacturing process origin inorganic salts—Being specified separately.

Water *<2.48>* Not more than 12.0% (10 mg, coulometric titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics *<4.02>* according to the following conditions. Perform the procedures of (iii), (iv) and (v) without exposure to direct or indirect sunlight.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Zinostatin Stimalamer RS equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration standard solution. Pipet 5 mL of the high concentration standard solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration standard solution.

(iv) Sample solutions—Weigh accurately an amount of Zinostatin Stimalamer equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration sample solution.

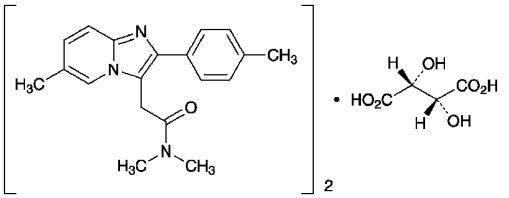
(v) Procedure—Allow to stand at 3 to 5°C for 2 hours before incubation.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding −20°C.

Zolpidem Tartrate

ゾルピデム酒石酸塩



$(C_{19}H_{21}N_3O)_2.C_4H_6O_6$: 764.87

N,*N*,6-Trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide hemi-(2*R*,3*R*)-tartrate

[*99294-93-6*]

Zolpidem Tartrate contains not less than 98.5% and not more than 101.0% of $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$, calculated on the anhydrous basis.

Description Zolpidem Tartrate occurs as a white, crystal-

line powder.

It is freely soluble in acetic acid (100), soluble in *N*,*N*-dimethylformamide and in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5) and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually changes to yellow in color on exposure to light.

Optical rotation $[\alpha]_D^{20}$: about +1.8° (1 g, *N*,*N*-dimethylformamide, 20 mL, 100 mm).

Identification **(1)** Dissolve 50 mg of Zolpidem Tartrate in 5 mL of acetic acid (100) and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Zolpidem Tartrate in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zolpidem Tartrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Zolpidem Tartrate in methanol (1 in 10) responds to the Qualitative Tests <*1.09*> (3) for tartrate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Zolpidem Tartrate 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—Dissolve 10 mg of Zolpidem Tartrate in 20 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peak other than the peak of zolpidem from the sample solution is not larger than the peak area of zolpidem from the standard solution.

Operating conditions—

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

Column: A stainless steel tube 4.6 mm in inside diameter and 7.5 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: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust the pH to 5.5 with triethylamine. To 11 volumes of this solution add 5 volumes of methanol and 4 volumes of acetonitrile.

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

Time span of measurement: About 5 times as long as the retention time of zolpidem.

System suitability—

System performance: Dissolve 10 mg each of Zolpidem Tartrate and benzyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 5 μL of this solution under the above operating conditions, zolpidem and benzyl parahydroxybenzoate 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zolpidem is not more than 5.0%.

(3) Residual solvent Being specified separately.

Water <*2.48*> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Zolpidem Tartrate, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.24 mg of $(C_{19}H_{21}N_3O)_2.C_4H_6O_6$

Containers and storage Containers—Tight containers.

Storage conditions—Light-resistant.

Zolpidem Tartrate Tablets

ゾルピデム酒石酸塩錠

Zolpidem Tartrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6$: 764.87].

Method of preparation Prepare as directed under Tablets, with Zolpidem Tartrate.

Identification To 1 tablet of Zolpidem Tartrate Tablets add 100 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, and filter. Discard the first 20 mL of the filtrate, to a volume of the subsequent filtrate, equivalent to 1 mg of Zolpidem Tartrate according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 235 nm and 239 nm and between 292 nm and 296 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Zolpidem Tartrate Tablets add *V*/10 mL of 0.1 mol/L hydrochloric acid TS, and disintegrate the tablet by shaking for 15 minutes. Add 2*V*/5 mL of methanol, then add exactly *V*/10 mL of the internal standard solution, shake for 15 minutes, and add methanol to make *V* mL so that each mL contains about 0.1 mg of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water <*2.48*> in the same manner as

Zolpidem Tartrate), and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 25 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$
$= M_S \times Q_T/Q_S \times V/250$

M_S: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Zolpidem Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Zolpidem Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute 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 V mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 2.8 μg of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$ according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zolpidem tartrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Zolpidem Tartrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. Pipet 25 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 sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using diluted the 2nd fluid for dissolution test (1 in 2) as the blank.

Dissolution rate (%) with respect to the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/4$

M_S: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$ in 1 tablet

Assay To 20 Zolpidem Tartrate Tablets add $V/10$ mL of 0.1 mol/L hydrochloric acid TS, and disintegrate the tablet by shaking for 15 minutes. Add $2V/5$ mL of methanol, then add exactly $V/10$ mL of the internal standard solution, shake for 15 minutes, and add methanol to make V mL so that each mL contains about 1 mg of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$. Centrifuge this solution, add to 1 mL of the supernatant liquid add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Zolpidem Tartrate), and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 2.5 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and calculate the ratios, Q_T and Q_S, of the peak area of zolpidem to that of the internal standard.

Amount (mg) of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$ in 1 tablet of Zolpidem Tartrate Tablets
$= M_S \times Q_T/Q_S \times V/500$

M_S: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 100).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 7.5 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: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust to pH 5.5 with triethylamine. To 550 mL of this solution add 250 mL of methanol and 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of zolpidem 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, zolpidem 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of zolpidem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.