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 the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the 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 Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate 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
= 24.833 mg of $C_{14}H_{20}N_2O_2$

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

Pipemidic Acid Trihydrate

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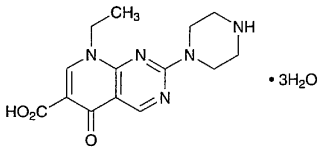

$C_{14}H_{17}N_5O_3.3H_2O$: 357.36
8-Ethyl-5,8-dihydro-5-oxo-2-(piperazin-1-yl)pyrido[2,3-*d*]pyrimidine-6-carboxylic acid trihydrate
[*51940-44-4*, anhydride]

Pipemidic Acid Trihydrate, when dried, contains not less than 98.5% of $C_{14}H_{17}N_5O_3$ (mol. wt.: 303.32).

Description Pipemidic Acid Trihydrate occurs as a pale yellow, crystalline powder. It is odorless, and has a bitter taste.

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

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.1 g of Pipemidic Acid Trihydrate in 20 mL of sodium hydroxide TS, heat under a reflux condenser in a water bath for 1 hour, and cool. To 2 mL of this solution add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, add 1 mL of dilute acetic acid, then add 4 mL of a solution of *p*-benzoquinone in methanol (1 in 1000), and boil gently: an orange-red color develops.

(2) Dissolve 0.1 g of Pipemidic Acid Trihydrate 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 the Ultraviolet-visible Spectrophotometry, 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 Pipemidic Acid Trihydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride—Dissolve 1.0 g of Pipemidic Acid Trihydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute nitric acid, 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—Dissolve 1.0 g of Pipemidic Acid Trihydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute hydrochloric acid, 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—Proceed with 2.0 g of Pipemidic Acid Trihydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

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

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Trihydrate 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 the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 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.

Loss on drying 14.5 - 16.0% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.3 g of Pipemidic Acid Trihydrate, previously dried, dissolve in 40 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L per-

chloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.332 mg of $C_{14}H_{17}N_5O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piperacillin Sodium

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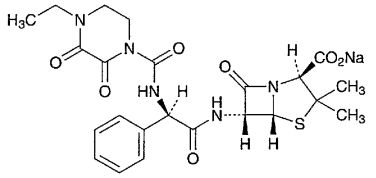

$C_{23}H_{26}N_5NaO_7S$: 539.54

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 ($C_{23}H_{27}N_5O_7S$: 517.56).

Description Piperacillin Sodium occurs as a white powder or mass.

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

Identification **(1)** Determine the infrared absorption spectrum of Piperacillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, 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 the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +175 - +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH 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—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—Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test using Apparatus B (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 this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate 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 of that of piperacillin from the standard solution, 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 sensitivity coefficinets, 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 (%)	Mobile phase B (%)
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 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 steps and the symmetry coefficient 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%.