

(2) Heavy metals—Proceed with 1.0 g of Netilmicin 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 0.05 g of Netilmicin Sulfate, calculated on the dried basis, in water to make 5 mL, and use this solution as the sample solution. Pipet 0.5 mL, 1 mL, and 1.5 mL of the sample solution, add water to each to make exactly 50 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 the Thin-layer Chromatography. Spot 5  $\mu$ L each of the sample solution and the standard solution (1), the standard solution (2), the standard solution (3) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°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 (3), and the total amount of the intensity of the spots other than the principal spot from the sample solution is not more than 6%.

**Loss on drying** Not more than 15.0% (0.15 g, in vacuum not exceeding 0.67 kPa, 110°C, 3 hours). Sampling should be carried out in a manner to avoid moisture absorption.

**Residue on ignition** 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 according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium ii 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 7.8 to 8.0 after sterilization.

(3) Standard solution—Weigh accurately an amount of Netilmicin Sulfate Reference Standard equivalent to about 0.025 g (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 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 4  $\mu$ g (potency) and 1  $\mu$ g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

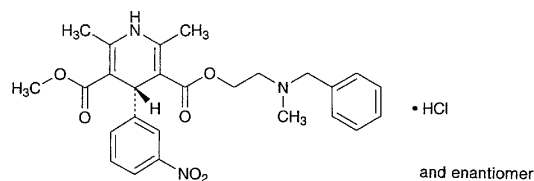
(4) Sample solution—Weigh accurately an amount of Netilmicin Sulfate equivalent to about 0.025 g (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4  $\mu$ g (potency) and 1  $\mu$ g (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, not exceeding 5°C, under nitrogen or argon atmosphere.

## Nicardipine Hydrochloride

塩酸ニカルジピン



$C_{26}H_{29}N_3O_6 \cdot HCl$ : 515.99

2-(*N*-Benzyl-*N*-methylamino)ethyl methyl (*RS*)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate monohydrochloride [54527-84-3]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of  $C_{26}H_{29}N_3O_6 \cdot HCl$ .

**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 the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, 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.

(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 for chloride.

**Melting point** 167 – 171°C

**Purity (1)** Heavy metals—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 10  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography 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 from the sample solution is not more than twice 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  $\mu$ m 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 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  $\mu$ L of this solution is equivalent to 8 to 12% of that of nicardipine obtained from 10  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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** Not more than 1.0% (1 g, 105°C, 2 hours).

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

**Assay** Conduct this procedure without exposure to day-light, 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 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<sub>26</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>·HCl

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

Storage—Light-resistant.

## Nicardipine Hydrochloride Injection

塩酸ニカルジピン注射液

Nicardipine Hydrochloride Injection is an aqueous solution for injection. It contains not less than 93%

and not more than 107% of the labeled amount of nicardipine hydrochloride (C<sub>26</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>·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 the Ultraviolet-visible Spectrophotometry: it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

**pH** 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 10  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography 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 more than the peak area of nicardipine from the standard solution, and the total of the areas of the peaks other than nicardipine from the sample solution is not more 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 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  $\mu$ L of this solution is equivalent to 8 to 12% of that from the standard solution.

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

**System repeatability:** When the test is repeated 5 times with 10  $\mu$ L 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** Less than 8.33 EU/mg.

**Assay** Conduct the procedure without exposure to day-light using light-resistant vessels. To an exact volume of Nicardipine Hydrochloride Injection, equivalent to about 2 mg of nicardipine hydrochloride (C<sub>26</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>·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 0.05 g of nicardipine hydrochloride for assay, previously dried at 105°C for 2