Document QAS/04.084/FINAL
February 2005

WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

NELFINAVIR MESILATE
Final text for inclusion in *The International Pharmacopoeia*

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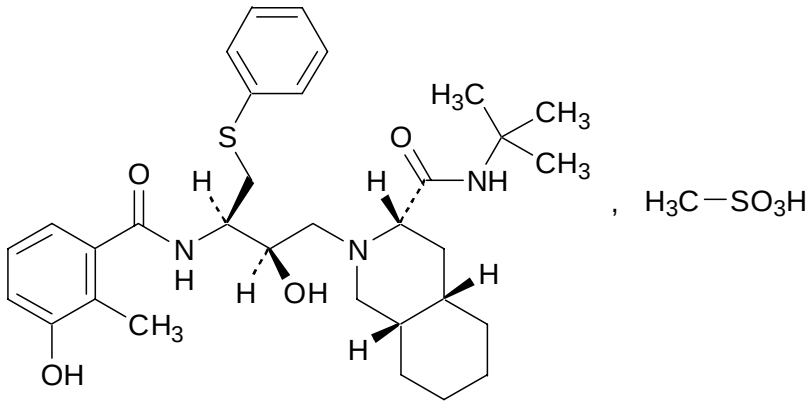

$C_{32}H_{45}N_3O_4S,CH_4O_3S$

Relative molecular mass. 663.9

Chemical name. (3*S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)-2-[(2*R*,3*R*)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylsulfanyl)butyl]decahydroisoquinoline-3-carboxamide methanesulfonate; CAS reg. No. 159989-65-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Nelfinavir mesilate should be kept in a tightly closed container, protected from light.

Additional information. Nelfinavir mesilate is hygroscopic.

Requirements

Nelfinavir mesilate contains not less than **98.5%** and not more than **101.0%** of $C_{32}H_{45}N_3O_4S,CH_4O_3S$, calculated with reference to the dried substance.

Identity tests

- ***Either tests A and B or test C may be applied.***

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing(A) 1 mg of the test substance per ml and (B) 1 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with basic potassium permanganate (5 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 40 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits a maximum at about 253 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 124 to136.

C. Carry out the examination as described under ‘‘Spectrophotometry in the infrared region’’ (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from nelfinavir mesilate RS or with the *reference spectrum* of nelfinavir mesilate.

Specific optical rotation. Use a 10.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = -105° to -125 °.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under ‘‘Limit test for heavy metals’’, Procedure 2, (Vol. 1, p.118); determine the heavy metals content according to Method A (Vol. 1, p.119); not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 100 °C; it loses not more than 30 mg/g.

Related substances. Carry out the test as described under “High–performance liquid chromatography” (Vol. 5, p. 257), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5µm)[1].

Use the following conditions for gradient elution:

Mobile phase A: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of purified water.

Mobile phase B: 41 volumes of acetonitrile R, 31 volumes of methanol R and 28 volumes of phosphate buffer pH 3.4.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute it to 1000 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-27	100	0	Isocratic
27-60	100 to 0	0 to 100	Linear gradient
60-75	0	100	Isocratic
75-80	0 to 100	100 to 0	Return to the initial conditions
80-90	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10.0 µg of Nelfinavir mesilate per ml. For solution (3) use 100 µg of methanesulfonic acid R per ml.

For the system suitability test: prepare solution (4) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 225 nm.

Maintain the column at 35 °C using, for example, a water-bath.

[1] HYPERSIL BDS C18 is suitable.

Inject 20 µl of solution (4). The test is not valid unless the resolution factor between the principal peak (retention time = about 27 minutes) and the peak with a retention time relative to the principal peak of about 0.2 is not less than 15. The test is also not valid unless the resolution factor between the last two peaks out of three peaks, which increase during the decomposition process, is not less than 4.0. The ratio of the retention times of these two peaks relative to the principal peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile R in both mobile phases A and B, or adjust the gradient program.

Inject 20 µl of solution (3).

Inject alternatively 20 µl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of related substances as a percentage. In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than that obtained with solution (2) (0.5 %). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (1.0 %). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3).

Assay. Dissolve about 0.50 g, accurately weighed, in 50 ml of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Perform a blank determination and make the necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 66.39 mg of $C_{32}H_{45}N_3O_4S.CH_4O_3S$.

Impurities *Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.*

Reagents

Silica gel for chromatography, octadecylsilyl, base-deactivated
A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimise the interaction with basic compounds.

Methanesulfonic acid R

Molecular formula. CH_4O_3S

Description. Colourless and corrosive liquid, strong irritant.

Solubility. Miscible with water.

Density (d). ~1.48.

Melting point. About 20 °C.

Potassium permanganate, basic (5 g/l) TS

A solution of potassium permanganate R containing about 5 g of $KMnO_4$ per litre of sodium hydroxide (1 mol/l).

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