Nelfinavir mesilate oral powder (Nelfinaviri mesilas pulvis peroralum)

Category. Antiretroviral (Protease Inhibitor).

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

Labelling. The designation on the container of nelfinavir mesilate oral powder should state that the active ingredient is in the mesilate form, and the quantity should be indicated in terms of the equivalent amount of nelfinavir.

Requirements

Complies with the monograph for Oral Powders.

Definition. Nelfinavir mesilate oral powder contains Nelfinavir mesilate. It contains not less than 90.0% and not more than 110.0% of the amount of nelfinivir (C\textsubscript{32}H\textsubscript{45}N\textsubscript{3}O\textsubscript{4}S) stated on the label.

Identity tests

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 1.14.1 Thin-layer chromatography 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 the following 2 solutions in methanol R: (A) shake a quantity of the oral powder equivalent to about 21 mg of nelfinavir with 5 mL, filter and use the clear filtrate; and (B) 5 mg of nelfinavir mesilate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or 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 to that obtained with solution B.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Spray the plate 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 to that obtained with solution B.

B. To a quantity of the oral powder equivalent to about 20 mg of nelfinavir add 50 mL of methanol R, shake and filter. Dilute 5 mL of the filtrate to 50 mL with the same solvent. The absorption spectrum (1.6) of the resulting solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 253 nm.

Related substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of base-deactivated silica gel, the surface of which has been modified by chemically-bonded octadecylsilyl groups (5 µm).

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 water R.

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 R in 800 mL of water R, adjust the pH to 3.4 by adding phosphoric acid (~105 g/l) TS and dilute it to 1000 mL with water R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–27</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>27–60</td>
<td>100 to 0</td>
<td>0–100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>60–75</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>
For solution (1) mix and transfer a quantity of the oral powder equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 mL volumetric flask. Add about 20 mL of methanol R, sonicate for about 15 minutes, allow to cool to room temperature and make up to volume using mobile phase A. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. For solution (2) dilute a suitable volume of solution A to obtain a concentration equivalent to 10.0 µg of nelfinavir per mL of mobile phase A. For solution (3) use 100 µg of methanesulfonic acid per mL of mobile phase A.

For the system suitability test: prepare solution (4) using 2 mL of solution (1) and 5 mL of sulfuric acid (~440 g/l) TS, 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 225 nm. Maintain the column at 35 °C.

Inject 20 µl of solution (4). The test is not valid unless (1) the resolution between the principal peak (retention time about 27 minutes) and the peak with a relative retention time of about 0.2 is at least 15 and (2) the resolution between the last two peaks out of three peaks, that increase during decomposition, is at least 4.0. (The relative retention of these two peaks is about 1.8 and 1.9, respectively.) If necessary adjust the amount of acetonitrile in both mobile phases A and B or adjust the gradient programme.

Inject 20 µl of solution (3).

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

In the chromatogram obtained with solution (1) the area of any peak, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). The area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of not more than three such peaks is greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). The sum of the areas of all peaks, other than the principal peak, is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0 %). Disregard any peak with a retention time less than 5 minutes and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %). Disregard any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3). The area of any peak, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%), the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of not more than three such peaks is greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Assay

Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of base-deactivated silica gel, the surface of which has been modified by chemically-bonded octadecylsilyl groups (5 µm).

As the mobile phase use a solution prepared as follows: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of water R. Prepare the phosphate buffer by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 3.4 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 mL with water R.

For solution (1) mix and transfer a quantity of the oral powder equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 mL volumetric flask. Add about 20 mL of methanol, sonicate for about 15 minutes, allow to cool to room temperature and make up to volume using the mobile phase. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. For solution (2) use 2.3 mg of nelfinavir mesilate RS per mL prepared in the same manner.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 225 nm. Maintain the column temperature at 35 °C.

Inject 20 µl of solution (2) in six replicate injections into the chromatographic system. The assay is not valid unless the relative standard deviation for the peak area of nelfinavir is less than 2.0 %.

Inject alternatively 20 µl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of nelfinavir.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of nelfinavir (C_{32}H_{45}N_{3}O_{4}S).
B. Mix and transfer a quantity of the oral powder equivalent to about 20 mg of nelfinavir, accurately weighed, to a 50 mL volumetric flask. Add about 25 mL of methanol R, sonicate for about 5 minutes, allow to cool to room temperature and make up to volume using the same solvent. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. Dilute 5.0 mL of the filtrate to 50.0 mL with the same solvent. Measure the absorbance of this solution in a 1 cm layer at the maximum at about 253 nm against a solvent cell containing methanol R.

Calculate the content of nelfinavir \((C_{32}H_{45}N_{3}O_{4}S)\) using an absorptivity value of 15.7 \(\text{mol}^{-1}\text{cm}^{-1}\) = 157. \[
\text{Absorbance} = \frac{A_{1%}^{1\%}}{\text{Concentration}}
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