

BRIEFING

Nelfinavir Tablets. This monograph was posted on the USP Non-US Monographs Web page as Draft 1 on August 27, 2010, and has been available for review and public comment for more than 90 days. No comments were received. The Small Molecules 1 Expert Committee has reviewed the draft and approved the monograph as an Authorized Non-US Monograph. The HPLC procedures in the test for *Organic Impurities* and in the *Assay* are based on analyses performed with the Hypersil BDS C18 brand of L1 column. The typical retention times of nelfinavir in the *Assay* and in the test for *Organic Impurities* are about 6 min and about 19 min, respectively.

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Nelfinavir Tablets

v.1 Authorized March 1, 2011

DEFINITION

Nelfinavir Tablets contain an amount of nelfinavir mesylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of nelfinavir (C₃₂H₄₅N₃O₄S).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**
Standard solution: 1.2 mg/mL of USP Nelfinavir Mesylate RS in methanol
Sample solution: 1 mg/mL of nelfinavir in methanol from finely powdered Tablets (NLT 20). [NOTE—Sonicate to dissolve if necessary.]
Application volume: 5 µL
Developing solvent system: Dichloromethane, acetonitrile, methanol, and ammonia (67:20:10:3)
Analysis
Samples: *Standard solution* and *Sample solution*
 When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Evaluate the spots under UV light at 254 nm.
Acceptance criteria: The *R_F* value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **NELFINAVIR**
Buffer: Dissolve 4 g of monobasic sodium phosphate dihydrate and 1 g of octanesulfonic acid sodium salt in 1000 mL of water. Add 1.0 mL of dimethylamine.
Mobile phase: Acetonitrile, methanol, and *Buffer* (9:4:7)
Diluent: Methanol and water (3:2)
Standard stock solution: 1.2 mg/mL of USP Nelfinavir Mesylate RS in *Diluent*. [NOTE—Sonicate to dissolve if necessary.]
Standard solution: 0.12 mg/mL of USP Nelfinavir Mesylate RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 1 mg/mL of nelfinavir from finely powdered Tablets (NLT 20). Dissolve initially with 0.1 N hydrochloric acid using 8% of the final volume, and sonicate for 5 min. Add *Diluent* using 60% of the final volume, and sonicate again for 15 min. Cool the solution to room temperature, and dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size.
Sample solution: 0.1 mg/mL of nelfinavir in *Mobile phase* from the *Sample stock solution*
Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 2 mL/min
Injection size: 10 µL
Run time: 1.5 times the retention time of nelfinavir
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of the labeled amount of nelfinavir (C₃₂H₄₅N₃O₄S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U* = peak response of nelfinavir from the *Sample solution*
 - r_S* = peak response of nelfinavir from the *Standard solution*
 - C_S* = concentration of USP Nelfinavir Mesylate RS in the *Standard solution* (mg/mL)
 - C_U* = nominal concentration of nelfinavir in the *Sample solution* (mg/mL)
 - M_{r1}* = molecular weight of nelfinavir, 567.78
 - M_{r2}* = molecular weight of nelfinavir mesylate, 663.89
- Acceptance criteria:** 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm, with sinker
Time: 45 min
Standard solution: USP Nelfinavir Mesylate RS in *Medium* at a concentration similar to the one expected in the *Sample solution*. Methanol can be used to help solubilize nelfinavir.
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute with *Medium* if necessary.
Analytical wavelength: UV 250 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of the labeled amount of nelfinavir (C₃₂H₄₅N₃O₄S) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

- A_U* = absorbance of the *Sample solution*
- A_S* = absorbance of the *Standard solution*
- C_S* = concentration of USP Nelfinavir Mesylate RS in the *Standard solution* (mg/mL)
- L* = label claim (mg/Tablet)
- M_{r1}* = molecular weight of nelfinavir, 567.78
- M_{r2}* = molecular weight of nelfinavir mesylate, 663.89
- V* = volume of *Medium*, 900 mL
- D* = dilution factor of the *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of nelfinavir is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
Mobile phase: Proceed as directed in the *Assay*.
Standard stock solution: 1.2 mg/mL of USP Nelfinavir Mesylate RS dissolved initially, using 10% of the final volume, with methanol. Dilute with *Mobile phase* to final volume.
Standard solution: 0.012 mg/mL of USP Nelfinavir Mesylate RS in *Mobile phase* from the *Standard stock solution*
Sample solution: 1 mg/mL of nelfinavir from finely powdered Tablets (NLT 20) in *Mobile phase*. Sonicate for 5 min, and cool the solution to room temperature before diluting with *Mobile*

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phase to volume. Pass the solution through a suitable filter of 0.45- μm pore size.

Sensitivity solution: 1.2 $\mu\text{g}/\text{mL}$ of USP Nelfinavir Mesylate RS in *Mobile phase* from the *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 10 μL

Run time: 3 times the retention time of nelfinavir

System suitability

Samples: *Standard stock solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates for the nelfinavir peak, *Standard stock solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.0% for the nelfinavir peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nelfinavir Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of nelfinavir from the *Standard solution*

C_s = concentration of USP Nelfinavir Mesylate RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of nelfinavir in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nelfinavir, 567.78

M_{r2} = molecular weight of nelfinavir mesylate, 663.89

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 1.5%

SPECIFIC TESTS

- **WATER DETERMINATION, Method Ia (921):** NMT 9.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Nelfinavir Mesylate RS

(3*S*,4*aS*,8*aS*)-*N*-*tert*-Butyl-2-[(2*R*,3*R*)-3-(3,2-cresotamido)-2-hydroxy-4-(phenylthio)butyl]decahydro-3-isoquinolinecarboxamide monomethanesulfonate (salt).

$\text{C}_{32}\text{H}_{45}\text{N}_3\text{O}_4\text{S} \cdot \text{CH}_4\text{O}_3\text{S}$ 663.89