

BRIEFING

**Nelfinavir Oral Powder.** 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 *Dissolution* test and in the *Assay* are based on analyses performed with the Inertsil ODS brand of L1 column. The typical retention time of nelfinavir in the *Assay* is about 8 min. The HPLC procedures in the test for *Organic Impurities* are based on analyses performed with the Hypersil BDS C18 brand of L1 column. The typical retention time of nelfinavir in the test for *Organic Impurities* is about 31 min.

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**Nelfinavir Oral Powder**

v.1 Authorized March 1, 2011

**DEFINITION**

Nelfinavir Oral Powder contains an amount of nelfinavir mesylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of nelfinavir (C<sub>32</sub>H<sub>45</sub>N<sub>3</sub>O<sub>4</sub>S).

**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. Sonicate for 5–10 min until the sample dissolves, cool, and dilute with methanol to volume.  
**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<sub>F</sub> value and intensity of the principal spot of the *Sample solution* correspond to those 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.  
**Diluent:** Methanol and water (7:3)  
**Mobile phase:** Acetonitrile, methanol, and *Buffer* (9:4:7)  
**Standard stock solution:** 1.2 mg/mL of USP Nelfinavir Mesylate RS in *Diluent*. [NOTE—Sonicate to dissolve if necessary.]  
**Standard solution:** 0.24 mg/mL of USP Nelfinavir Mesylate RS from the *Standard stock solution*. Dilute by adding 0.1 N hydrochloric acid, using 20% of the final volume, to an aliquot from the *Standard stock solution*, and then dilute with *Diluent* to volume.  
**Sample solution:** 0.2 mg/mL of nelfinavir. Dissolve initially with 0.1 N hydrochloric acid, using 20% of the final volume, and sonicate for about 10 min. Add *Diluent* using 40% of the final volume, and sonicate for 10 min. Cool the solution to room temperature, and dilute with *Diluent* to volume. Pass the solution through a 20-µm ashless filter paper, discarding the first few mL of filtrate, and use the remaining portion of the filtrate.

**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:** 40°  
**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**  
**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 (C<sub>32</sub>H<sub>45</sub>N<sub>3</sub>O<sub>4</sub>S) in the portion of Oral Powder taken:

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

- r<sub>U</sub> = peak response of nelfinavir from the *Sample solution*
  - r<sub>S</sub> = peak response of nelfinavir from the *Standard solution*
  - C<sub>S</sub> = concentration of USP Nelfinavir Mesylate RS in the *Standard solution* (mg/mL)
  - C<sub>U</sub> = nominal concentration of nelfinavir in the *Sample solution* (mg/mL)
  - M<sub>r1</sub> = molecular weight of nelfinavir, 567.78
  - M<sub>r2</sub> = 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:** 50 rpm  
**Time:** 45 min  
**Buffer, Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.  
**Standard stock solution:** USP Nelfinavir Mesylate RS in methanol  
**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration similar to that of the *Sample solution*.  
**Sample solution:** Transfer an accurately weighed portion of Oral Powder, equivalent to about 50 mg of nelfinavir, to the dissolution vessel. After the specified time, withdraw about 10 mL of the solution under test. Pass the solution through a 20-µm ashless filter paper, discarding the first few mL of filtrate, and use the remaining portion of the filtrate. Dilute with *Medium* if necessary.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of nelfinavir (C<sub>32</sub>H<sub>45</sub>N<sub>3</sub>O<sub>4</sub>S) dissolved:

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

- r<sub>U</sub> = peak response from the *Sample solution*
  - r<sub>S</sub> = peak response from the *Standard solution*
  - C<sub>S</sub> = concentration of nelfinavir mesylate in the *Standard solution* (mg/mL)
  - L = label claim (mg/mg of Oral Powder)
  - M<sub>r1</sub> = molecular weight of nelfinavir, 567.78
  - M<sub>r2</sub> = molecular weight of nelfinavir mesylate, 663.89
  - V = volume of *Medium*, 900 mL
  - D = dilution factor of the *Sample solution*
- Tolerances:** NLT 70% (Q) of the labeled amount of nelfinavir is dissolved.

**IMPURITIES**

- **ORGANIC IMPURITIES**  
**Buffer:** 4.9 g/L of anhydrous monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 3.4  
**Mobile phase:** Acetonitrile, methanol, *Buffer*, and water (27:20:28:25). Adjust with 0.1 N sodium hydroxide or phosphoric acid to a pH of 4.8.

2 / Nelfinavir

**Standard stock solution:** 0.12 mg/mL of USP Nelfinavir Mesylate RS. Dissolve using 5% of the final volume with methanol, and dilute with *Mobile phase* to volume.

**Standard solution:** 1.2 µg/mL of USP Nelfinavir Mesylate RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** 1 mg/mL of nelfinavir. Dissolve in methanol using 20% of the final volume, and sonicate for about 5 min. Add *Mobile phase* using 40% of the final volume, and sonicate for 5 min. Cool to room temperature, and dilute with *Mobile phase* to volume. Pass the solution through a suitable filter.

**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:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**Run time:** 3 times the retention time of nelfinavir

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 5.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Powder taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

- $r_u$  = 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
- $F$  = relative response factor for each impurity (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nelfinavir oxide <sup>a</sup>	0.35	0.67	0.5
Nelfinavir dioxide <sup>b</sup>	0.71	0.88	0.5

<sup>a</sup> (3S,4aS,8aS)-N-tert-Butyl-2-[(2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfinyl)butyl]decahydroisoquinoline-3-carboxamide.

<sup>b</sup> (3S,4aS,8aS)-N-tert-Butyl-2-[(2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfonyl)butyl]decahydroisoquinoline-3-carboxamide.

**Table 1** (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nelfinavir	1.0	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.5

<sup>a</sup> (3S,4aS,8aS)-N-tert-Butyl-2-[(2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfinyl)butyl]decahydroisoquinoline-3-carboxamide.

<sup>b</sup> (3S,4aS,8aS)-N-tert-Butyl-2-[(2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfonyl)butyl]decahydroisoquinoline-3-carboxamide.

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 100 cfu/g and the total yeast and molds count does not exceed 10 cfu/g. It meets the requirements of the tests for absence of *Escherichia coli*.
- **WATER DETERMINATION, Method Ia** (921): NMT 12.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers and protect from excessive moisture. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Nelfinavir Mesylate RS  
(3S,4aS,8aS)-N-tert-Butyl-2-[(2R,3R)-3-(3,2-cresotamido)-2-hydroxy-4-(phenylthio)butyl]decahydro-3-isoquinolinecarboxamide monomethanesulfonate (salt).  
C<sub>32</sub>H<sub>45</sub>N<sub>3</sub>O<sub>4</sub>S · CH<sub>4</sub>O<sub>3</sub>S 663.89