

BRIEFING

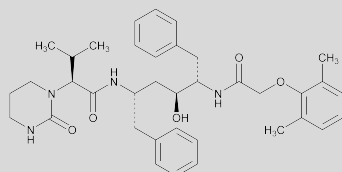
**Lopinavir.** A new USP Pending Monograph is being proposed based on submitted data. The HPLC procedure used in the test for *Organic Impurities* is based on analysis performed with the YMC Pack ODS-AQ brand of 5- $\mu$ m L1 column. The HPLC procedure used in the *Assay* is based on analysis performed with the Hypersil BDS C8 brand of 5- $\mu$ m L7 column. The typical retention time for lopinavir is about 24.5 min in the test for *Organic Impurities* and about 7 min for the *Assay*.

(MD-AA: L. Santos, B. Davani.) RTS—C65346

**Add the following:**

**Lopinavir**

Draft 1



$C_{37}H_{48}N_4O_5$  628.80  
[1*S*-[1*R*\*(*R*\*), 3*R*\*, 4*R*\*]]-*N*-[4[[2,6-Dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]-tetrahydro- $\alpha$ -(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide;  
( $\alpha$ *S*)-Tetrahydro-*N*-[( $\alpha$ *S*)- $\alpha$ -[(2*S*, 3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xyllyloxy)acetamido]butyl]phenethyl]- $\alpha$ -isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide [192725-17-0].

**DEFINITION**

Lopinavir contains NLT 98.0% and NMT 102.0% of  $C_{37}H_{48}N_4O_5$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the lopinavir peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- PROCEDURE**  
**Buffer:** 6.8 mg/mL of monobasic potassium phosphate. Adjust the pH to 3.0 with phosphoric acid. Pass the solution through a suitable (PTFE, PVDF, or equivalent) 0.45- $\mu$ m filter.  
**Mobile phase:** Acetonitrile, methanol, and *Buffer* (44:11:45)  
**Standard solution:** 0.2 mg/mL of USP Lopinavir RS in *Mobile phase*

**Sample solution:** 0.2 mg/mL in *Mobile phase*  
**Chromatographic system:** (See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 210 nm  
**Column:** 4.6-mm  $\times$  15-cm column; 5- $\mu$ m packing L7  
**Flow rate:** 1.5 mL/min  
**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*  
**Suitability requirements**  
**Column efficiency:** NLT 3000 theoretical plates  
**Tailing factor:** NMT 2.0  
**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{37}H_{48}N_4O_5$  in the portion of Lopinavir taken:

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

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of Lopinavir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

**IMPURITIES**

**Inorganic Impurities**

- RESIDUE ON IGNITION** (281): NMT 0.1%
- HEAVY METALS, Method II** (231): NMT 10 ppm

**Organic Impurities**

**PROCEDURE**

**Solution A:** 2.72 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5. Pass the solution through a suitable (PTFE, PVDF, or equivalent) 0.45- $\mu$ m filter.  
**Solution B:** Acetonitrile  
**Mobile phase:** See the gradient table below.

| Time (min) | Solution A (%) | Solution B (%) |
|------------|----------------|----------------|
| 0          | 60             | 40             |
| 30         | 35             | 65             |
| 40         | 20             | 80             |
| 50         | 20             | 80             |
| 52         | 60             | 40             |
| 60         | 60             | 40             |

**Diluent:** Acetonitrile and water (7:3)  
**System suitability solution:** 0.1 mg/mL of USP Lopinavir RS in *Diluent*  
**Standard solution:** 0.002 mg/mL of USP Lopinavir RS in *Diluent*  
**Sample solution:** 1.0 mg/mL of Lopinavir in *Diluent*  
**Chromatographic system** (See *Chromatography* (621), *System Suitability*.)

Mode: LC  
 Detector: UV 210 nm  
 Column: 4.6-mm × 25-cm column; 5-μm packing L1  
 Flow rate: 1 mL/min  
 Injection size: 20 μL  
 Column temperature: 45°

**System suitability**

**Sample:** *System suitability solution* and *Standard solution*  
 [NOTE—The relative retention times are listed in *Impurity Table 1*.]

**Suitability requirements**

**Column efficiency:** NLT 25000 theoretical plates, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for lopinavir, *Standard solution*

**Analysis**

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

Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

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

- $r_U$  = response of the impurity from the *Sample solution*  
 $r_S$  = response of lopinavir from the *Standard solution*  
 $C_S$  = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Lopinavir in the *Sample solution* (mg/mL)

**Impurity Table 1**

| Compound                                  | Relative Retention Time | Limit (%) |
|---|-------------------------|-----------|
| Lopinavir aminoalcohol <sup>1</sup>       | 0.15                    | 0.10      |
| Lopinavir phenoxyacetic acid <sup>2</sup> | 0.32                    | 0.10      |
| Lopinavir 4-hydroxy <sup>3</sup>          | 0.76                    | 0.30      |
| Lopinavir 4-oxo <sup>4</sup>              | 0.88                    | 0.30      |
| Lopinavir dimer <sup>5</sup>              | 0.93                    | 0.10      |
| Lopinavir                                 | 1.00                    | —         |
| Lopinavir isoleucine analog <sup>6</sup>  | 1.12                    | 0.10      |
| Lopinavir leucine analog <sup>7</sup>     | 1.16                    | 0.10      |
| Lopinavir diamide <sup>8</sup>            | 1.59                    | 0.10      |
| Lopinavir <i>N</i> -acyl <sup>9</sup>     | 1.68                    | 0.10      |
| Unidentified impurity                     | —                       | 0.10      |

<sup>1</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide pyro-L-glutamate.

<sup>2</sup>2-(2,6-Dimethylphenoxy)acetic acid.

<sup>3</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(4-hydroxy-2-oxotetrahydropyrimidin-1(2*H*)-yl)-3-methylbutanamide.

<sup>4</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-3-methylbutanamide.

<sup>5</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-4-Hydroxy-5-(2-{4-[2-((2*S*,3*S*,5*S*)-3-hydroxy-5-((*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido)-1,6-diphenylhexan-2-ylamino]-2-oxoethoxy}-3,5-dimethylphenyl)acetamido)-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

<sup>6</sup>(2*S*,3*S*)-*N*-[(2*S*,4*S*,5*S*)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)pentanamide.

<sup>7</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-4-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)pentanamide.

<sup>8</sup>*N,N'*-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

<sup>9</sup>(*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl]-3-methylbutanamide.

**Acceptance criteria**

**Total impurity:** NMT 1.0%

**SPECIFIC TESTS**

- OPTICAL ROTATION, Specific Rotation (7815):** −22° to −26°  
 Sample solution: 4 mg/mL in methanol
- WATER DETERMINATION, Method I (921):** NMT 4.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
 USP Lopinavir RS<sub>A</sub> (1-Sep-2009)