

BRIEFING

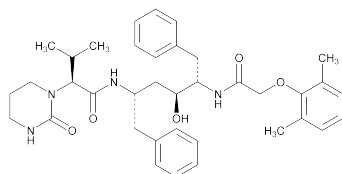
Lopinavir. This monograph has been posted on the USP Pending Standards Web page for review and public comment for 90 days. No comments were received. The MD-AA Expert Committee has approved the monograph as an Authorized USP Pending Standard.

The HPLC procedure used in the test for *Organic Impurities* is based on analysis performed with YMC Pack ODS-AQ brand of 5- μ m L1 column. The HPLC procedure used in the *Assay* is based on analysis performed with Hypersil BDS C8 brand of 5- μ m L7 column. The typical retention time for lopinavir is about 24.5 min in the test for *Organic Impurities* and about 7 min in the test for *Assay*.

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Lopinavir

v.1 Authorized September 1, 2009



$C_{37}H_{48}N_4O_5$ 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- α -(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide;
(α *S*)-Tetrahydro-*N*-[(α *S*)- α -[(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xilyloxy)acetamido]butyl]phenethyl]- α -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 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0. Pass the solution through a suitable 0.45- μ 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; 5- μ m packing L7
Flow rate: 1.5 mL/min
Injection size: 20 μ 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% on the anhydrous basis

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 0.45- μ 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 \times 25-cm; 5- μ m packing L1
Temperature: 45°
Flow rate: 1 mL/min
Injection size: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*
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:

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

2 / Lopinavir

- r_U = peak response of the impurity from the *Sample solution*
- r_S = peak 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

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lopinavir aminoalcohol ^a	0.15	0.10
Lopinavir phenoxyacetic acid ^b	0.32	0.10
Lopinavir 4-hydroxy ^c	0.76	0.30
Lopinavir 4-oxo ^d	0.88	0.30
Lopinavir dimer ^e	0.93	0.10
Lopinavir	1.00	—
Lopinavir isoleucine analog ^f	1.12	0.10
Lopinavir leucine analog ^g	1.16	0.10

^a (S)-N-[(2S,4S,5S)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide pyro-L-glutamate.

^b 2-(2,6-Dimethylphenoxy)acetic acid.

^c (S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(4-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)-3-methylbutanamide.

^d (S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-3-methylbutanamide.

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

^f (2S,3S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxotetrahydropyrimidin-1(2H)-yl)pentanamide.

^g (S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-4-methyl-2-(2-oxotetrahydropyrimidin-1(2H)-yl)pentanamide.

^h N,N'-[(2S,3S,5S)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

ⁱ (S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2H)-yl]-3-methylbutanamide.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lopinavir diamide ^h	1.59	0.10
LopinavirN-acyl ⁱ	1.68	0.10
Unidentified impurity	—	0.10

^a (S)-N-[(2S,4S,5S)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide pyro-L-glutamate.

^b 2-(2,6-Dimethylphenoxy)acetic acid.

^c (S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(4-hydroxy-2-oxotetrahydropyrimidin-1(2H)-yl)-3-methylbutanamide.

^d (S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-3-methylbutanamide.

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

^f (2S,3S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxotetrahydropyrimidin-1(2H)-yl)pentanamide.

^g (S)-N-[(2S,4S,5S)-5-(2-(2,6-dimethylphenoxy)acetamido)-4-hydroxy-1,6-diphenylhexan-2-yl]-4-methyl-2-(2-oxotetrahydropyrimidin-1(2H)-yl)pentanamide.

^h N,N'-[(2S,3S,5S)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

ⁱ (S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2H)-yl]-3-methylbutanamide.

Acceptance criteria

Total impurity: NMT 1.0%

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S):** -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