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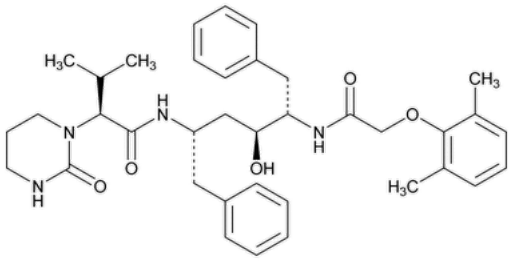
Lopinavir



$C_{37}H_{48}N_4O_5$ 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-xylyloxy)acetamido]butyl]phenethyl]-α-isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide CAS RN®: 192725-17-0; UNII: 2494G1JF75.

DEFINITION

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

IDENTIFICATION

Change to read:

• **A.** ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)

• **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: 2.7 g/L of monobasic potassium phosphate and 0.9 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0. Pass the solution through a suitable filter of 0.45-µm pore size.

Diluent: Acetonitrile and water (1:1)

Solution A: Acetonitrile and *Buffer* (9:11)

Mobile phase: *Solution A*

Standard solution: 0.025 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.025 mg/mL of Lopinavir in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 60 min

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 8000 theoretical plates

Capacity factor: NLT 15

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lopinavir ($C_{37}H_{48}N_4O_5$) 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 = concentration of Lopinavir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.2%

• **Organic Impurities: Procedure 1**

[Note—For early-eluting impurities.]

Buffer, Diluent, and **Solution A:** Prepare as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
60	100	0
61	0	100
81	0	100
82	100	0
100	100	0

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.5 mg/mL of Lopinavir in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 100 min

[Note—Data collection is only for the first 60 min. The remaining gradient steps wash out the late-eluting impurities and re-equilibrate the column.]

System suitability

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

[Note—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 1.2 between lopinavir *N*-formylphenoxyacetamide and lopinavir *N*-acetylphenoxyacetamide, *System suitability solution*

Capacity factor: NLT 15, *Standard solution*

Column efficiency: NLT 8000, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Diluent*, *System suitability solution*, *Standard solution*, and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each 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)

F = relative response factor (see *Table 2*)

Table 2

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir free amine[a]	0.03	0.61	0.1
Lopinavir *N*-formylaminoalcohol[b]	0.07	0.80	0.2
Lopinavir divalinate[c]	0.10	0.65	0.1
Sulfolopinavir[d]	0.13	0.76	0.1
Lopinavir phenoxyacetamide[e]	0.25	0.96	0.1
Lopinavir *N*-formylphenoxyacetamide[f]	0.59	1.3	0.1
Lopinavir *N*-acetylphenoxyacetamide[g]	0.62	1.2	0.1
Lopinavir oxazine[h]	0.90	1.1	0.1
Lopinavir	1.00	—	—
Isolopinavir[i]	1.10	0.99	0.2
Lopinavir 2,4-phenoxy isomer[j]	1.13	0.97	0.1
Lopinavir D-valine diastereomer[k]	1.25	1.1	0.1
Z-Diacylethenediamine[l]	1.28	1.4	0.1
Lopinavir (2*R*,4*R*) diastereomer[m]	1.32	1.0	0.1
Lopinavir (4*R*) epimer[n]	1.38	0.97	0.1
Any other individual impurity	—	1.0	0.1

[a] (*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.

[b] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[c] (2*S*,2'*S*)-*N*,*N*'-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis{3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide}.

[d] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl hydrogen sulfate.

[e] *N*-[(2*S*,3*S*,5*S*)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

[f] 2-(2,6-Dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

[g] *N*-[(2*S*,3*S*,5*S*)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

[h] *N*-{(*S*)-1-[(4*S*,6*S*)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl}-2-(2,6-dimethylphenoxy)acetamide.

[i] (*S*)-*N*-{(2*S*,3*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[j] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[k] (*R*)-*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]butanamide.

[l] (*Z*)-*N*,*N*'-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

[m] (*S*)-*N*-{(2*R*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[n] (*S*)-*N*-{(2*S*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

• **Organic Impurities: Procedure 2**

[Note—For late-eluting impurities.]

Buffer, Diluent, and **Solution A:** Prepare as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: *Solution A* and *Solution B* (3:7)

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.5 mg/mL in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 50 min

System suitability

Sample: *Standard solution*

[Note—The relative retention times are listed in *Table 3*.]

Suitability requirements

Capacity factor: NLT 1.5

Column efficiency: NLT 3000

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Diluent*, *System suitability solution*, *Standard solution*, and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each 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)

F = relative response factor (see *Table 3*)

Table 3

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir	1.00	—	—
Lopinavir *O*-acyl[a]	1.49	0.77	0.1
Lopinavir (2*R*) epimer[b]	1.91	1.1	0.1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir diamide[c]	4.39	1.4	0.1
Lopinavir *N*-acyl[d]	6.01	1.3	0.1
Lopinavir *O*-phenoxyacetyl[e]	7.14	1.1	0.1
Lopinavir amino alcohol urea[f]	8.46	1.3	0.1
Any other individual impurity	—	1.0	0.1
Total impurities from Procedure 1 and Procedure 2	—	1.0	0.7[g]

[a] (*S*)-{(2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanamido]-1,6-diphenylhexan-3-yl} 3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate.

[b] (*S*)-*N*-{(2*R*,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]butanamide.

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

[d] (*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.

[e] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl 2-(2,6-dimethylphenoxy)acetate.

[f] *N*,*N*'-(2*S*,2'*S*,3*S*,3'*S*,5*S*,5'*S*)-5,5'-Carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

[g] Exclude from *Organic Impurities, Procedure 2*, lopinavir (4*R*) epimer and any other peak eluting prior to this peak because these are already monitored in *Procedure 1*.

Acceptance criteria: See *Table 2* and *Table 3*.

SPECIFIC TESTS

• **Water Determination,** *Method I*⟨921⟩**:** NMT 4.4%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at room temperature.

• **USP Reference Standards** ⟨11⟩

USP Lopinavir RS

USP Lopinavir System Suitability Mixture RS

Lopinavir System Suitability Mixture contains lopinavir *N*-formylphenoxyacetamide, lopinavir *N*-acetylphenoxyacetamide, and several other minor components.

Lopinavir *N*-formylphenoxyacetamide is (2-(2,6-dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide. $C_{29}H_{34}N_2O_4$ 474.59

Lopinavir *N*-acetylphenoxyacetamide is (*N*-[(2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide. $C_{30}H_{36}N_2O_4$ 488.62

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
LOPINAVIR	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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