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Lopinavir and Ritonavir Tablets

DEFINITION

Lopinavir and Ritonavir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Lopinavir and Ritonavir**

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer 1* (50:50)

Buffer 2: 2.1 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile and 1-butanol (13:3)

Solution C: Acetonitrile, 1-butanol, *Buffer 1*, and water (65:15:10:10)

Standard solution: 6.25 µg/mL of USP Ritonavir RS and 25 µg/mL of USP Lopinavir RS in *Solution A*

Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir in a 250-mL volumetric flask, add 25 mL of *Buffer 2*, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution B*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and then further dilute with *Solution A* to a nominal concentration of 6.25 µg/mL of ritonavir and 25 µg/mL of lopinavir.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer 1* (175:100:100:625)

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[Note—The elution order is ritonavir, then lopinavir.]

Suitability requirements

Capacity factor: 15–24 for the ritonavir peak

Tailing factor: 0.8–1.2 for the ritonavir peak

Theoretical plates: More than 5000 for the ritonavir peak

Relative standard deviation: NMT 2.0% for the ritonavir and lopinavir peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Tablets taken:

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

r_U = peak response of lopinavir or ritonavir from the *Sample solution*

r_S = peak response of lopinavir or ritonavir from the *Standard solution*

C_S = concentration of lopinavir or ritonavir in the *Standard solution* (µg/mL)

C_U = nominal concentration of lopinavir or ritonavir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$)

PERFORMANCE TESTS

• **Dissolution ⟨711⟩**

Medium: 60 mM polyoxyethylene 10 lauryl ether (37.56 g/L) in water; 900 mL

Apparatus 2: 75 rpm

Time: 90 min

Mobile phase: Acetonitrile and 4.1 g/L potassium phosphate monobasic (55:45). Adjust with phosphoric acid to an apparent pH of 4.0 ± 0.05.

Standard solution: Dissolve USP Lopinavir RS in methanol to obtain a solution containing 2.6 mg/mL. Dissolve USP Ritonavir RS in methanol to obtain a solution containing 1.3 mg/mL. Combine portions of these solutions to make a solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir in *Medium*.

Sample solutions: Pass a portion of the solution under test through a suitable filter. If necessary, dilute the solution with *Medium* to obtain a final sample solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir.

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between lopinavir and ritonavir

Tailing factor: 0.9–1.5 for the lopinavir and ritonavir peaks

Relative standard deviation: NMT 2.0% for the lopinavir and ritonavir peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved:

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

r_U = peak response of lopinavir or ritonavir from the *Sample solution*

r_S = peak response of lopinavir or ritonavir from the *Standard solution*

C_S = concentration of USP Lopinavir RS or USP Ritonavir RS in the *Standard solution* (mg/mL)

L = Tablet label claim for lopinavir or ritonavir (mg)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) are dissolved.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water

Solution A: *Buffer 1* and acetonitrile (50:50)

Buffer 2: 2.1 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile, 1-butanol, and *Buffer 1* (15:5:80)

Solution C: Acetonitrile, 1-butanol, *Buffer 1*, and water (65:15:10:10)

Solution D: Acetonitrile and 1-butanol (13:3)

Buffer solution: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate in water

Mobile phase: Acetonitrile, tetrahydrofuran,1-butanol, and *Buffer solution* (18:8:5:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to a pH of 6.3 ± 0.1.

Standard stock solution: 0.025 mg/mL of USP Ritonavir RS in *Solution A*

Standard solution: 2.5 µg/mL of USP Ritonavir RS in *Solution B* from *Standard stock solution*

Ritonavir degradant identification solution: Transfer two 5.0 mL portions of a 1 mg/mL solution of USP Ritonavir RS in *Solution A* to separate 50-mL volumetric flasks. Add 1 g of citric acid to one flask, and shake until dissolved. Heat both flasks at 80° for approximately 24 h. Cool the flasks, and add 13 mL of 1 N sodium hydroxide to the flask containing the citric acid. Dilute both flasks with *Solution B* to volume. Combine equal volumes of both solutions. This solution contains ritonavir and the ritonavir degradation products (*N*-deacylvaline ritonavir, hydantoin aminoalcohol, *O*-acyl isomer, and oxazolidinone derivative).

Ritonavir related compounds identification solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS dissolved in *Solution C* and further diluted with *Solution B* to 0.5 mg/mL.

Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir into a 250-mL volumetric flask. Add 25 mL of *Buffer 2*, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution D*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and further dilute with *Solution B* to a concentration of 2 mg/mL of lopinavir and 0.5 mg/mL of ritonavir.

Chromatographic system

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

Column: 4.6-mm × 15-cm; 3-µm packing L26

Column temperature: 60°

Detector: UV 240 nm

Injection volume: 50 µL

Flow rate: 1.0 mL/min

System suitability

Samples: *Ritonavir degradant identification solution, Ritonavir related compounds identification solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the peaks for *O*-acyl isomer and oxazolidinone derivative, *Ritonavir degradant identification solution.* NLT 0.7 between the peaks for hydroxyritonavir and hydantoin aminoalcohol, *Ritonavir related compounds identification solution*

Capacity factor: NLT 10.8, *Standard solution*

Tailing factor: 0.8–1.2, *Standard solution*

Column efficiency: NLT 5000, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each ritonavir degradation product in the *Sample solution*:

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

r_U = peak area of individual degradation product from the *Sample solution*

r_S = peak response of ritonavir from the *Standard solution*

C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

F = relative response factor

Acceptance criteria: See *Table 1*. [NOTE—Disregard all peaks eluting before the retention time of the *N*-deacylvaline ritonavir peak from the *Ritonavir degradant identification solution*.]

Table 1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Deacylvaline ritonavir[a]	0.11	0.81	0.2

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetamidoalcohol[b]	0.15	—	—[*]
2,5-Thiazolylmethyl dicarbamate[c]	0.24	—	—[*]
Hydroxyritonavir[d]	0.36	0.86	0.3
Hydantoin aminoalcohol[e]	0.39	0.73	2.6
Ritonavir hydroperoxide[f]	0.44	0.88	0.2
Hydantoin-oxazolidinone derivative[g]	0.50	—	—[*]
Ethyl analog[h]	0.64	—	—[*]
O-Acyl isomer[i]	0.74	1.1	0.2
BOC-aminoalcohol[j]	0.81	—	—[*]
Isobutoxycarbonyl aminoalcohol[k]	0.81	—	—[*]
Oxazolidinone derivative[l]	0.87	0.53	0.3
Ureidovaline isobutyl ester[m]	0.94	—	—[*]
Ritonavir	1.0	—	—[*]
4-Hydroxy isomer[n]	1.05	—	—[*]
3*R*-Epimer[o]	1.11	—	—[*]
Aminoalcohol urea derivative[p]	1.14	—	—[*]
3*R*,5*R*-Epimer[q]	1.23	—	—[*]
5*R*-Epimer[r]	1.32	—	—[*]
Diacyl valine urea[s]	1.70	—	—[*]
Any unspecified impurity	—	1.0	0.2[**]
Total impurities	—	—	3.5[**]

* Process impurities; for information only.

** Disregard any peak less than 0.05%.

[a] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[b] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[c] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.

[d] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[g] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(S)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.

[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[i] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[k] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[l] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.

[m] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

[n] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[o] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[p] Bis(thiazol-5-ylmethyl) (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)dicarbamate.

[q] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[r] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[s] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

ADDITIONAL REQUIREMENTS

- **USP Reference Standards** ⟨11⟩

USP Lopinavir RS
USP Ritonavir RS
USP Ritonavir Related Compounds Mixture RS

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

Topic/Question	Contact	Expert Committee
LOPINAVIR AND RITONAVIR TABLETS	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

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