Ritonavir Capsules

Type of Posting	Revision Bulletin
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Expert Committee	Chemical Medicines Monographs 1
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 1 Expert Committee has revised the Ritonavir Capsules monograph. The purpose for the revision is to add *Dissolution Test 2* for a drug product approved by the FDA with different dissolution conditions and tolerances than the existing dissolution tests. *Labeling* information has been incorporated to support the inclusion of *Dissolution Test 2*.

- *Dissolution Test 2* was validated using a Kromasil C8 brand of L7 column. The typical retention time for ritonavir is about 3.9 min.

In addition, the term “disregard limit” in the acceptance criteria of the test for *Organic Impurities* has been replaced with “reporting threshold” in the acceptance criteria of the test for *Organic Impurities* to be consistent with current *USP* style.

The revision also necessitates a change in the table numbering in the *Organic Impurities* section.

The Ritonavir Capsules Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in *USP 42–NF 37*.

Should you have any questions, please contact Shankari Shivaprasad, Ph.D., Senior Scientific Liaison (301-230-7426 or sns@usp.org).

Ritonavir Capsules

DEFINITION

Ritonavir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 4.1 g/L of monobasic potassium phosphate
Diluent: Acetonitrile and *Buffer* (50:50)
Mobile phase: Acetonitrile, methanol, tetrahydrofuran (stabilizer-free), and *Buffer* (7:4:4:25). Separately filter the *Buffer* and the pre-mixed solvents before combining to make the *Mobile phase*.
Standard solution: 25 µg/mL of USP Ritonavir RS in *Diluent*
Sample stock solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer Capsules (NLT 5) equivalent to 500 mg of ritonavir into a 500-mL volumetric flask, add about 250 mL of *Diluent*, and shake for at least 30 min or until the Capsules have visually disintegrated. Add 150 mL of acetonitrile, allow to cool to room temperature, and dilute to volume with *Diluent*.
Sample solution: Nominally 25 µg/mL of ritonavir in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 240 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*
Suitability requirements
Capacity factor: NLT 15
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Capsules 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 Ritonavir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲**Test 1**▲ (RB 1-Apr-2018)
Medium: 0.1 N hydrochloric acid with 25 mM polyoxyethylene 10 lauryl ether, 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 30 min
Buffer: 4.1 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (55:45). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Standard stock solution: 5.2 mg/mL of USP Ritonavir RS in methanol
Standard working solution: 104 µg/mL of USP Ritonavir RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 240 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
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)
L = ritonavir label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) is dissolved.
▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid with 25 mM polyoxyethylene 10 lauryl ether; 900 mL, degassed
Apparatus 2: 50 rpm, with sinker
Time: 20 and 120 min
Solution A: Water and phosphoric acid (98:2)
Buffer: Water adjusted with *Solution A* to a pH of 3.5
Mobile phase: Acetonitrile, methanol, and *Buffer* (500:100:400).
Standard stock solution: 0.56 mg/mL of USP Ritonavir RS in methanol
Standard solution: 0.11 mg/mL of USP Ritonavir RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
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 concentration (C_i) of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the sample withdrawn from the vessel at each time point (*i*):

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

r_U = peak response of ritonavir 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)

Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of ritonavir in the portion of sample withdrawn at the specified time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of ritonavir (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point *i* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (min)	Tolerances (*Q*)
1	20	20%–40%
2	120	NLT 80%▲ (RB 1-Apr-2018)

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

IMPURITIES

Change to read:

• **Organic Impurities**

[NOTE—Ritonavir is alkali sensitive. All glassware should be pre-rinsed with distilled water before use to remove residual detergent contamination.]

Buffer A: 4.1 g/L of monobasic potassium phosphate
Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate
Solution A: Acetonitrile and *Buffer A* (50:50)
Solution B: Acetonitrile and *Buffer A* (65:35)
Solution C: Butyl alcohol and *Buffer A* (8:92)
Mobile phase: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer B* (18:5:8:69). Adjust apparent pH to 6.3 ± 0.1 with 1 M phosphoric acid or 1 M potassium hydroxide if necessary.
Cleaning solution: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer A* (30:8:13:49)
Standard stock solution: 0.1 mg/mL of USP Ritonavir RS in *Solution A*
Standard solution: 10 µg/mL of USP Ritonavir RS in *Solution C* from *Standard stock solution*
Peak identification solution: Transfer 5–10 g from contents of Capsules into a suitable sealed container. Add an amount of citric acid equivalent to 1% of the Capsule weight taken, and mix until dissolved. Seal the container, and heat at 60° for about 24 h. Transfer about 2 g to a 100-mL volumetric flask, and dilute with *Solution B* to volume. Transfer 5.0 mL of the solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, and seal the tube with a stopper. Shake vigorously until a uniform emulsion is obtained, making sure to vent periodically. The emulsion formed yields distinct layers when centrifuged. The top layer (clear heptane) and the bottom layer (clear sample solution) are separated by a viscous white cloudy layer. The middle layer is part of the heptane layer. Carefully remove the clear heptane layer and the middle layer. Pass the bottom layer through a solid phase extraction cartridge containing strong anion-exchange packing in acetate form as described below.
Sample stock solution: Nominally 2 mg/mL of ritonavir prepared as follows. Empty the contents of Capsules (NLT 6) into a suitable container, and accurately weigh and transfer an equivalent to 200 mg of ritonavir to a 100-mL volumetric flask. Dissolve and dilute with *Solution B* to volume.
Sample solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer 25.0 mL of *Sample stock solution* into a 50-mL volumetric flask, and dilute with *Solution C* to volume. Add 15.0 mL of this solution into a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, and seal the tube with a stopper. Shake vigorously until a uniform emulsion is obtained, making sure to vent periodically. The emulsion formed yields distinct layers when centrifuged. The top layer (clear heptane) and the bottom layer (clear sample solution) are separated by a viscous white cloudy layer. The middle layer is part of the heptane layer. Carefully remove the clear heptane layer and the middle layer. Pass the bottom layer through a solid phase extraction cartridge containing strong anion-exchange packing in acetate form as described below. Condition a solid phase extraction cartridge with methanol and *Solution B* two separate times, and dry for 10 min under low vacuum. Add 5.0 mL of the clear sample solution into the reservoir. Collect the sample solution at a slow rate into a 5-mL volumetric flask using low vacuum. Dilute with *Solution B* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3-µm packing L26. Wash the column after each injection of the *Peak identification solution* and each injection of the *Sample solution* with *Cleaning solution* for about 26 min, and equilibrate with *Mobile phase* for about 30 min. Store in *Cleaning solution* after the analysis is completed.
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 1.8 times the retention time of ritonavir
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: NLT 13
Tailing factor: 0.8–1.2
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules 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 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 (see ▲*Table 2*)▲ (RB 1-Apr-2018)

Acceptance criteria: See ▲*Table 2*. Reporting threshold is▲ (RB 1-Apr-2018) 0.05%.

▲Table 2▲ (RB 1-Apr-2018)

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline[a, b]	0.03	—	—
N-Deacylvaline ritonavir[c, d]	0.11	1.0	0.4
Acetamidoalcohol[b, e]	0.15	1.0	0.1
2,5-Thiazolylmethyldicarbamate[b, f]	0.24	1.37	0.1
Hydroxyritonavir[d, g]	0.36	1.0	0.2
Hydantoin aminoalcohol[d, h]	0.39	0.73	0.9
Ritonavir hydroperoxide[d, i]	0.44	—	—
Ethanol adduct[d, j]	0.45	0.66	0.3
Hydantoin-oxazolidinone derivative[b, k]	0.50	0.76	0.2
Ethyl analog[b, l]	0.64	1.0	0.1
Geo-isomer[d, m]	0.74	1.0	0.3
BOC-aminoalcohol[b, n]		—	—
Isobutoxycarbonyl aminoalcohol[b, o]	0.81	0.74	0.1
Oxazolidinone derivative[d, p]	0.87	0.53	1.0
Ureidovaline isobutyl ester[b, q]	0.94	1.0	0.1
Ritonavir	1.00	—	—
4-Hydroxy isomer[b, r]	1.05	1.0	0.1
3*R*-Epimer[b, s]	1.11	1.0	0.3
Aminoalcohol urea derivative[b, t]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[b, u]	1.23	1.0	0.1
5*R*-Epimer[b, v]	1.32	1.0	0.1
Diacyl valine urea[b, w]	1.70	1.0	0.1
Any other individual impurity	—	—	0.2
Total process impurity	—	—	0.8
Total impurities	—	—	3.0

[a] [*N*-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine (not quantified by this method due to solvent front and placebo interferences).
[b] Process impurity.
[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[d] Degradation impurity.
[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[f] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.
[g] 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.
[h] 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.
[i] 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 (report as ethanol adduct due to possible co-elution).
[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[k] (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.
[l] 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.
[m] (*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.
[n] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may co-elute with isobutoxycarbonyl aminoalcohol; report as isobutoxycarbonyl aminoalcohol).
[o] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.
[p] (*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.
[q] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.
[r] 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.
[s] 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.
[t] 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.
[u] 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.
[v] 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.
[w] (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.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 2° and 8°.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Apr-2018)

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ritonavir RS