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## 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  $\mu$ L**System suitability****Sample:** Standard solution**Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** Standard solution and Sample solutionCalculate 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):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

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

 $C_i$  = 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 ▲ (ERR 1-Nov-2018)
1	20	20%–40%
2	120	NLT 80% ▲(Q) ▲ (ERR 1-Nov-2018)

▲ (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  $6.3 \pm 0.1$  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 <sup>a,b</sup>	0.03	—	—
N-Deacylvaline ritonavir <sup>c,d</sup>	0.11	1.0	0.4
Acetamidoalcohol <sup>b,e</sup>	0.15	1.0	0.1
2,5-Thiazolylmethyldicarbamate <sup>b,f</sup>	0.24	1.37	0.1
Hydroxyritonavir <sup>d,g</sup>	0.36	1.0	0.2
Hydantoin aminoalcohol <sup>d,h</sup>	0.39	0.73	0.9
Ritonavir hydroperoxide <sup>d,i</sup>	0.44	—	—
Ethanol adduct <sup>d,j</sup>	0.45	0.66	0.3
Hydantoin-oxazolidinone derivative <sup>b,k</sup>	0.50	0.76	0.2
Ethyl analog <sup>b,l</sup>	0.64	1.0	0.1
Geo-isomer <sup>d,m</sup>	0.74	1.0	0.3
BOC-aminoalcohol <sup>b,n</sup>	0.81	—	—
Isobutoxycarbonyl aminoalcohol <sup>b,o</sup>		0.74	0.1
Oxazolidinone derivative <sup>d,p</sup>	0.87	0.53	1.0
Ureidovaline isobutyl ester <sup>b,q</sup>	0.94	1.0	0.1
Ritonavir	1.00	—	—
4-Hydroxy isomer <sup>b,r</sup>	1.05	1.0	0.1
3R-Epimer <sup>b,s</sup>	1.11	1.0	0.3
Aminoalcohol urea derivative <sup>b,t</sup>	1.14	1.0	0.1
3R,5R-Diastereomer <sup>b,u</sup>	1.23	1.0	0.1
5R-Epimer <sup>b,v</sup>	1.32	1.0	0.1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Diacyl valine urea <sup>b,w</sup>	1.70	1.0	0.1
Any other individual impurity	—	—	0.2
Total process impurity	—	—	0.8
Total impurities	—	—	3.0

<sup>a</sup> [N-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine (not quantified by this method due to solvent front and placebo interferences).

<sup>b</sup> Process impurity.

<sup>c</sup> Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

<sup>d</sup> Degradation impurity.

<sup>e</sup> Thiazol-5-ylmethyl (2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

<sup>f</sup> Bis(thiazol-5-ylmethyl) (2S,3S,5S)-3-hydroxy-1,6-diphenylhexane-2,5-diylidicarbamate.

<sup>g</sup> Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-2-(3-[(2-(2-hydroxypropan-2-yl)thiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

<sup>h</sup> Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

<sup>i</sup> Thiazol-5-ylmethyl (2S,3S,5S)-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).

<sup>j</sup> Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

<sup>k</sup> (4S,5S)-Thiazol-5-ylmethyl 4-benzyl-5-[(S)-2-[(S)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate.

<sup>l</sup> Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

<sup>m</sup> (S)-{(2S,3S,5S)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanoate.

<sup>n</sup> Thiazol-5-ylmethyl (2S,3S,5S)-(5-t-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may co-elute with isobutoxycarbonyl aminoalcohol; report as isobutoxycarbonyl aminoalcohol).

<sup>o</sup> Thiazol-5-ylmethyl (2S,3S,5S)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

<sup>p</sup> (S)-N-[(S)-1-[(4S,5S)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamide.

<sup>q</sup> (S)-Isobutyl 2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanoate.

<sup>r</sup> Thiazol-5-ylmethyl (2S,4S,5S)-4-hydroxy-5-[(S)-2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

<sup>s</sup> Thiazol-5-ylmethyl (2S,3R,5S)-3-hydroxy-5-[(S)-2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

<sup>t</sup> Bis(thiazol-5-ylmethyl) (2S,2'S,3S,3'S,5S,5'S)-5,5'-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

<sup>u</sup> Thiazol-5-ylmethyl (2S,3R,5R)-3-hydroxy-5-[(S)-2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

<sup>v</sup> Thiazol-5-ylmethyl (2S,3S,5R)-3-hydroxy-5-[(S)-2-3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

<sup>w</sup> (3S,4S,6S,10S,13S,15S,16S)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetraabenzy-2,7,9,12,17-pentaazaocadecanedioate.

#### 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](#)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
RITONAVIR CAPSULES	<a href="#">Documentary Standards Support</a>	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	SM12020 Small Molecules 1

**Chromatographic Database Information:** [Chromatographic Database](#)

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