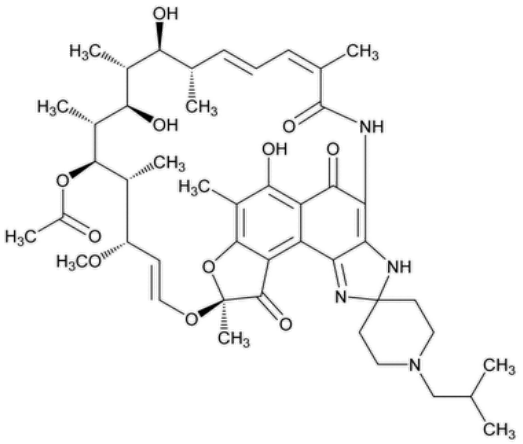
Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Oct-2022
Document Type: USP Monographs
DocId: GUID-872712CE-CB73-45C3-9CDE-835E2A910E58_6_en-US
DOI: https://doi.org/10.31003/USPNF_M73600_06_01
DOI Ref: 3wl74

Rifabutin

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-rifabutin-20220930 .



$C_{46}H_{62}N_4O_{11}$ 847.02

(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-Trihydroxy-1′-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethyl-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphtho[1,2-*d*]imidazole-2,4′-piperidin]-16-yl acetate CAS RN®: 72559-06-9.

DEFINITION

Rifabutin contains NLT 950 µg/mg and NMT 1020 µg/mg of rifabutin ($C_{46}H_{62}N_4O_{11}$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K
- **B.** 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**

Solution A: 13.6 g/L of monobasic potassium phosphate

Mobile phase: Acetonitrile and *Solution A* (50:50). Adjust by dropwise addition of 2 N sodium hydroxide to a pH of 6.5 ± 0.1.

System suitability solution: Dissolve about 10 mg of Rifabutin in 2 mL of methanol, add 1 mL of 2 N sodium hydroxide, and allow to stand for 4 min. Add 1 mL of 2 N hydrochloric acid, and dilute with *Mobile phase* to 50 mL. [Note—Portions of this solution may be stored in the frozen state for future use.]

Standard solution: 0.5 mg/mL of USP Rifabutin RS prepared as follows. Transfer an amount of USP Rifabutin RS to a suitable volumetric flask. Add acetonitrile to fill 10% of the volume of the flask, and dilute with *Mobile phase* to volume.

Sample solution: 0.5 mg/mL of Rifabutin prepared as follows. Transfer an amount of Rifabutin to a suitable volumetric flask. Add acetonitrile to fill 10% of the volume of the flask, and dilute with *Mobile phase* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 12.5-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of the rifabutin peak

System suitability

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

[NOTE—The chromatogram of the *System suitability solution* exhibits a major peak for a degradant, two minor peaks for degradants, and a major peak for rifabutin at relative retention times of about 0.5, 0.6, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.3 between the degradant peak eluting at a relative retention time of about 0.8 and the rifabutin peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of rifabutin ($C_{46}H_{62}N_4O_{11}$) in the portion of Rifabutin taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

P = potency of USP Rifabutin RS (µg/mg)

Acceptance criteria: 950–1020 µg/mg on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1: LIMIT OF *N*-ISOBUTYLPIPERIDONE**

Diluent: Chloroform and methanol (1:1)

Standard solution 1: 0.1 mg/mL of *N*-isobutylpiperidone in *Diluent*

Standard solution 2: 0.05 mg/mL of *N*-isobutylpiperidone in *Diluent*

Standard solution 3: 0.02 mg/mL of *N*-isobutylpiperidone in *Diluent*

Standard solution 4: 0.01 mg/mL of *N*-isobutylpiperidone in *Diluent*

Standard solution 5: 0.005 mg/mL of *N*-isobutylpiperidone in *Diluent*

Sample solution: 10 mg/mL of Rifabutin in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Hexanes and acetone (100:30)

Analysis: Apply portions of the *Sample solution* and the *Standard solutions* to a suitable TLC plate (see Chromatography ⟨621⟩) coated with *Adsorbent*. Develop the chromatograms in the *Developing solvent system*, contained in an equilibrated unlined chromatographic chamber, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, allow the plate to air-dry, and place it in an iodine vapor chamber until the spots are visible (about 5 min). Remove the plate from the chamber, spray the plate with starch TS, and examine the plate.

Acceptance criteria: NMT 0.5%. No spot from the *Sample solution* at an R_F value corresponding to that of *N*-isobutylpiperidone is more intense than that of the principal spot observed from *Standard solution 2*.

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: 0.1% (v/v) triethylamine in water

Solution B: Acetonitrile and *Solution A* (20:80). Adjust with trifluoroacetic acid to a pH of 2.5.

Solution C: Acetonitrile and *Solution A* (90:10). Adjust with trifluoroacetic acid to a pH of 2.5.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	75	25
35	75	25
45	45	55
50	40	60
60	30	70
70	30	70
71	75	25

Diluent: Acetonitrile and *Solution A* (20:80). Adjust with trifluoroacetic acid to a pH of 6.0.

System suitability solution: Dissolve 10 mg of Rifabutin in 2 mL of methanol, add 1 mL of 2 N sodium hydroxide, and allow to stand for 4 min. Add 1 mL of 2 N hydrochloric acid, and dilute with *Diluent* to 50 mL. [NOTE—Portions of this solution may be stored in the frozen state for future use.]

Standard stock solution: 1 mg/mL of USP Rifabutin RS prepared as follows. Transfer an amount of USP Rifabutin RS to a suitable volumetric flask and add acetonitrile to fill 10% of the final volume of the flask. Dilute with *Diluent* to volume.

Standard solution: 0.01 mg/mL of USP Rifabutin RS in *Diluent* from *Standard stock solution*

Sensitivity solution: 0.5 µg/mL of USP Rifabutin RS in *Diluent* from *Standard solution*

Sample solution: 1 mg/mL of Rifabutin prepared as follows. Transfer an amount of Rifabutin to a suitable volumetric flask. Add acetonitrile to fill 10% of the final volume of the flask, and dilute with *Diluent* to volume. [NOTE—Prepare the solution just before the injection.]

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between the rifabutin 14*R* enantiomer and rifabutin peaks, *System suitability solution*

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

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Rifabutin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

P = potency of USP Rifabutin RS (µg/mg)

F_1 = conversion factor (0.001 mg/µg)

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16-Desacetylrifabutin[a]	0.45	1.0	1.0
Rifabutin 14*R* epimer[b]	0.86	1.0	0.75
Rifabutin	1.0	1.0	—
Rifabutin *N*-oxide[c]	1.2	1.0	0.50
Rifabutin 21*R* epimer[d]	1.25	1.0	0.75
Didehydrorifabutin[e]	1.50	1.32	1.0
Rifabutin *N*-oxide open ring[f]	1.60	1.0	0.25
Specified unknown impurity	1.93	1.0	0.50
Any unspecified impurity	—	1.0	▲0.5▲ (RB 1-Oct-2022)
Total impurities	—	—	3.0

[a] (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,16,18,20-Tetrahydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2',3':7,8]naphtho[1,2-*d*]imidazole-2,4'-piperidine]-5,10,26(3*H*,9*H*)-trione.

[b] (9*S*,12*E*,14*R*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-Trihydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethyl-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2',3':7,8]naphtho[1,2-*d*]imidazole-2,4'-piperidine]-16-yl acetate.

[c] (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-16-Acetyloxy-6,18,20-trihydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2',3':7,8]naphtho[1,2-*d*]imidazole-2,4'-piperidine]-5,10,26(3*H*,9*H*)-trione 1'-oxide.

[d] (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*R*,22*E*,24*Z*)-6,18,20-Trihydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethyl-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2',3':7,8]naphtho[1,2-*d*]imidazole-2,4'-piperidine]-16-yl acetate.

[e] (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-Trihydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethyl-21-methylene-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2',3':7,8]naphtho[1,2-*d*]imidazole-2,4'-piperidine]-16-yl acetate.

[f] (11*R*,13*E*,16*S*,17*R*,18*S*,19*R*,20*R*,21*S*,22*S*,23*E*,25*Z*)-17-Acetyloxy-6,8,11,19,21-pentahydroxy-15-methoxy-7,11,16,18,20,22,26-heptamethyl-1'-(2-methylpropyl)-5,10,27-trioxo-3,5-dihydrospiro[4,9-(epiminopentadeca[2,4,14]trienoxyethano)naphtho[1,2-*d*]imidazole-2,4'-piperidine] $N^{1'}$-oxide.

SPECIFIC TESTS

• **Water Determination ⟨921⟩, *Method I*:** NMT 2.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light and from excessive heat.

• **USP Reference Standards ⟨11⟩**

USP Rifabutin RS

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

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

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 44(4)

Current DocID: GUID-872712CE-CB73-45C3-9CDE-835E2A910E58_6_en-US

DOI: https://doi.org/10.31003/USPNF_M73600_06_01

DOI ref: 3wl74