RIFAMPICIN

(RIFAMPICINUM)

Draft proposal for revision for The International Pharmacopoeia

(June 2022)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to Dr Herbert Schmidt, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (email: schmidt@who.int), with a copy to Ms Sinéad Jones (email: jonessi@who.int) by 2 September 2022.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the “Working documents in public consultation” link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/20.866:

RIFAMPICIN

(RIFAMPICINUM)

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<td>Proposal drafted following discussions with the WHO Prequalification Programme.</td>
<td>November 2020</td>
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<td>Draft proposal sent out for public consultation.</td>
<td>November 2020 - January 2021</td>
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<td>Discussion at the Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.</td>
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[Note from the Secretariat. Following the determination of traces of nitrosamines in some batches of rifampicin active pharmaceutical ingredient, it is proposed to revise the corresponding monograph.]

The revision is based on information found in other pharmacopoeias and in the public domain and on laboratory investigations.

Changes to the current chapter are indicated in the text by insert or delete.]
Rifampicin (Rifampicinum)

Molecular formula. $C_{43}H_{58}N_4O_{12}$

Relative molecular mass. 822.9.

Graphic formula

Chemical name. $(2S,12Z,14E,16S,17S,18R,19R,20R,21S,22R,23S,24E)-5,6,9,17,19$-
pentahydroxy-$23$-methoxy-$2,4,12,16,18,20,22$-heptamethyl-$8$-[[$(4$-methylpiperazin-$1$-
yl)imino]$methyl]-$1,11$-dioxo-$1,2$-dihydro-$2,7$-
(epoxypentadeca[$1,11,13$]trienimino)naphtho[$2,1-b$]furan-$21-yl$ acetate; $3$-[[$(4$-methyl-

Other name. Rifampin.

Description. A reddish-brown or brownish-red brick red to red-brown, crystalline
powder.

Solubility. Very slightly to slightly soluble in water R; soluble in methanol R; slightly
soluble in acetone R and ethanol ($\sim$750 g/L) TS; and ether R.

Category. Antileprosy; antituberculosis medicine.
Storage. Rifampicin should be kept in a tightly closed container or in an atmosphere of nitrogen, protected from light.

Additional information. Rifampicin exhibits polymorphism.

Requirements

Definition. Rifampicin contains not less than 97.0% and not more than 102.0% of C_{43}H_{58}N_{4}O_{12}, calculated with reference to the dried substance.

Manufacture. The production method is validated to demonstrate to the requirements of the responsible regulatory authority that the suspected carcinogenic nitrosamine 1-nitroso-4-methyl piperazine (MeNP) is eliminated or minimized and adequately controlled in the final product. A suitable method to determine MeNP in Rifampicin active pharmaceutical ingredient or Rifampicin tablets can be found in the Supplementary Section of The International Pharmacopoeia under Test methods used during development or manufacture.

[Note from the Secretariat. The mentioned test method to determine MeNP is described in document QAS 21-899, which currently also undergoes public consultation.]

Identity tests

• Either test A or any two of tests B, C or D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from rifampicin RS or with the reference spectrum of rifampicin.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and rifampicin RS in a small amount of dichloromethane R and evaporating to dryness. The infrared
absorption spectrum is concordant with the spectrum obtained from rifampicin RS.

B. Dissolve 50 mg of the test substance in 50 mL of methanol R and dilute 1 mL of this solution to 50 mL with phosphate buffer, pH 7.4, TS. The absorption spectrum of the resulting solution, when observed between 220 nm and 500 nm, exhibits 4 maxima at about 237 nm, 254 nm, 334 nm and 475 nm; the ratio of the absorbance of a 1 cm layer at the maximum at about 334 nm to that at the maximum at about 475 nm is about 1.75.

C. Carry out the test as described under 1.14.1 Chromatography. Thin-layer chromatography using silica gel R6 as the coating substance and a freshly prepared mixture of ethyl acetate R, ammonia (~260 g/L) TS, ethanol (~750 g/L) TS and cyclohexane R (20:9:4.5:5 V/V) as the mobile phase. Apply separately to the plate 10 µL of each of the following 2 solutions in methanol R containing (A) 1.0 mg of the test substance per mL and (B) 1.0 mg of rifampicin RS per mL. Develop the plate. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to rifampicin in the chromatogram obtained with solution (B).

D. Suspend 25 mg of the test substance in 25 mL of water R, shake for 5 minutes and filter. To 5 mL of the filtrate, add 1 mL of ammonium persulfate/phosphate buffer TS and shake for a few minutes; the colour turns from orange-yellow to violet-red without the formation of a precipitate.

Heavy metals (2.2.3). [Note from the Secretariat. Regarding the need to comply with the test for heavy metals, kindly see the corresponding note in chapter 2.2.3] Place 1.0 g in a silica crucible and mix it with 4 mL of magnesium sulfate/sulfuric acid TS. Heat cautiously to ignition and continue heating until a white or, at most, greyish residue is obtained. Ignite at a temperature not exceeding 800 °C, allow to cool, and moisten the
residue with a few drops of sulfuric acid (~100 g/L) TS. Evaporate, ignite again, and allow to cool. Next, dissolve the residue in hydrochloric acid (~70 g/L) TS, add, drop by drop, a solution of ammonia (~100 g/L) PbTS, until the pH of the solution is between 8 and 8.5, then add, also drop by drop, acetic acid (~60 g/L) PbTS to adjust the pH to 3–4, filter, dilute with water to 40 mL and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 μg/g.

Sulfated ash (2.3). Not more than 1.0 mg/g, determined on 2.0 g.

Loss on drying. Dry 1.000 g of the test substance at 80–60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 10 mg/g.

pH value(1.13). Shake 0.10 g with 10 mL of carbon-dioxide-free water R; pH of the suspension, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Chromatography. High-performance liquid chromatography, using a stainless steel column (4.6 mm x 15 cm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 µm).¹

As the mobile phase, use a mixture of 35 volumes of acetonitrile R and 65 volumes of a solution containing 0.1 % (V/V) of phosphoric acid (~1440 g/L) TS, 1.9 g/L of sodium perchlorate R, 5.9 g/L of citric acid R and 20.9 g/L of potassium dihydrogen phosphate R.

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Prepare as a solvent solution a mixture of 10 volumes of a 210.1 g/L solution of citric acid R, 23 volumes of a 136.1 g/L solution of potassium dihydrogen phosphate

¹ A Lichrosorb RP8 or Nucleosil C8 were found suitable.
R, 77 volumes of a 174.2 g/L solution of dipotassium hydrogen phosphate R, 250 volumes of acetonitrile R and 640 volumes of water R.

Prepare the following solutions immediately before use: for solution (1), dissolve 20.0 mg of the test substance in acetonitrile R and dilute to 10.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with the solvent solution. For solution (2), dissolve 30.0 mg of rifampicin quinone RS (impurity A) in acetonitrile R and dilute to 100.0 mL with the same solvent. To 1.0 mL of this solution, add 1.0 mL of solution (1) and dilute to 100.0 mL with the solvent mixture. For solution (3) [0.05%], dilute 5.0 mL of solution (2) to 100.0 mL.

Inject 20 µL each of solutions (1), (2) and (3). Record the chromatogram for about twice the retention time of rifampicin.

Use the chromatogram obtained with solution (2) to identify the peak due to impurity A.

The test is not valid unless, in the chromatogram obtained with solution (2), the resolution between the peaks due to rifampicin and impurity A is at least 4.0. The test is also not valid unless the signal-to-noise ration of the peak due to rifampicin obtained with solution (3) is at least 10.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, is not greater than 1.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (1.5 %);
- the area of any other impurity peak is not greater than the area of the peak due to rifampicin in the chromatogram obtained with solution (2) (1.0 %).
- The sum of the areas of all impurity peaks, other than impurity A, is not greater than 3.5 times the area of the peak due to rifampicin in the chromatogram obtained with solution (2) (3.5%). Disregard any peak with an
area of less than the area of the peak due to rifampicin in the chromatogram obtained with solution (3) (0.05%).

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and preparing the slurry with phosphate/citrate buffer pH 6.0, TS. As the mobile phase, use a mixture of 85 volumes of chloroform R and 15 volumes of methanol R. Apply separately to the plate 20 μl of each of 4 solutions in chloroform R containing (A) 20 mg of the test substance per mL, (B) 0.10 mg of 3-formylrifamycin SV RS per mL, (C) 0.30 mg of rifampicin quinone RS per mL and (D) 0.20 mg of the test substance per mL. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in daylight. Any coloured spots obtained with solution A, other than the principal spot, are not more intense than the corresponding spots obtained with solutions B and C. Any other spots obtained with solution A are not more intense than that obtained with solution D.

Assay. Dissolve about 0.100 g, accurately weighed, in sufficient methanol R and dilute to produce 100.0 mL with the same solvent. Dilute 2.0 mL of this solution to 100.0 mL with phosphate buffer, pH 7.4, TS. Measure the absorbance of the resulting solution as described under 1.6 Spectrophotometry in the visible and ultraviolet regions in a cuvette or cell with an optical pathlength of 10 mm 1-cm layer at the maximum at about 475 nm, using as the blank phosphate buffer, pH 7.4, TS. Calculate the content of C₄₃H₅₈N₄O₁₂ using the absorptivity value of 18.7 (A₁_cm = 187).

Impurities
A. Rifampicin quinone

B. Rifampicin N-oxide

**Reagents to be added**

**Sodium perchlorate R**

NaClO₄·H₂O; Molecular weight 140.5; CAS Reg. No. 7791-07-3.

**Content.** Not less than 99.0% of NaClO₄·H₂O.

**Description.** White or almost white, deliquescent crystals, very soluble in water.

**Storage.** Store in a well-closed container.

**Reference substances evoked**

**Rifampicin RS**
International Chemical Reference Substance (ICRS) available.

Rifampicin quinone RS

International Chemical Reference Substances (ICRS) available.

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