Status: Currently Official on 16-Feb-2025
Official Date: Official Prior to 2013
Document Type: USP Monographs
DocId: GUID-A710DEC0-2A70-4BEC-B0EC-9EA9808E103D_1_en-US
DOI: https://doi.org/10.31003/USPNF_M73660_01_01
DOI Ref: y3xrj

Rifampin and Isoniazid Capsules

» Rifampin and Isoniazid Capsules contain not less than 90.0 percent and not more than 130.0 percent of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoniazid ($C_6H_7N_3O$).

[Note—Where Rifampin and Isoniazid Capsules are prescribed without reference to the quantity of rifampin or isoniazid contained therein, a product containing 300 mg of rifampin and 150 mg of isoniazid shall be dispensed.]

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—

USP Rifampin RS
USP Isoniazid RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution—Transfer a portion of Capsule contents, equivalent to about 120 mg of rifampin, to a suitable flask, add 20 mL of methanol, and shake for several minutes. Pass this suspension through a filter having a 1-µm or finer porosity, discarding the first few mL of the filtrate. Dilute a volume of the filtrate with an equal volume of acetone, and mix.

Standard solutions—Dissolve a quantity of USP Rifampin RS in methanol to obtain a solution containing 6 mg per mL. Add an equal volume of acetone, and mix. Dissolve a quantity of USP Isoniazid RS in methanol to obtain a solution containing 2.5 mg per mL. Add an equal volume of acetone, and mix.

Application volume: 2 µL.

Developing solvent solution: a mixture of acetone and glacial acetic acid (100:1).

B: The retention times of the rifampin and isoniazid peaks in the chromatogram of the *Assay preparation* correspond to those of rifampin and isoniazid in the chromatogram of the *Standard preparation,* as obtained in the *Assay for rifampin and isoniazid.*

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Determine the amount of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved by employing the following method.

Phosphate buffer solution—Dissolve 15.3 g of dibasic potassium phosphate and 80.0 g of monobasic potassium phosphate into a 1-L volumetric flask, mix, dilute with water to volume, and mix.

Isoniazid standard solution—Accurately weigh about 66 mg of USP Isoniazid RS into a 100-mL volumetric flask. Dissolve in and dilute with 0.1 N hydrochloric acid to volume, and mix.

Standard stock solution—Accurately weigh about 66 mg of USP Rifampin RS into a 200-mL volumetric flask, dissolve in 10 mL of 0.1 N hydrochloric acid, and mix. Add 50.0 mL of *Isoniazid standard solution,* dilute with 0.1 N hydrochloric acid to volume, and mix. [Note—Prepare this solution immediately before the test is performed, and place in the dissolution bath at the start of the test.]

Standard solution—At the end of the test run, transfer a 5.0-mL aliquot of the *Standard stock solution* and 10.0 mL of *Phosphate buffer solution* to a 50-mL volumetric flask. Dilute with water to volume, and mix. [Note—Analyze the solution immediately, if possible, and if not, within 3 hours after final dilution.]

Test solution—At the end of the test run, withdraw a 25-mL aliquot, and filter, discarding the first 10 mL of the filtrate. Allow to cool for about 10 minutes, and transfer 5.0 mL of the filtrate and 10.0 mL of the *Phosphate buffer solution* to a 50-mL volumetric flask. Dilute with water to volume, and mix. [Note—Analyze the solution immediately, if possible, and if not, within 3 hours after final dilution.]

Determine the amount of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved from absorbances at the wavelength of maximum absorbance at about 475 nm of the *Standard solution* and the *Test solution.*

Determine the amount of isoniazid ($C_6H_7N_3O$) dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, *Phosphate buffer solution,* and methanol (850:100:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the isoniazid peaks.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{43}H_{58}N_4O_{12}$ and not less than 80% *(Q)* of the labeled amount of $C_6H_7N_3O$ are dissolved in 45 minutes.

Loss on drying ⟨731⟩—Dry about 100 mg of Capsule contents in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay for rifampin and isoniazid—

Buffer solution—Dissolve 1.4 g of dibasic sodium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 6.8.

Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (96:4).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (55:45).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Rifampin RS and USP Isoniazid RS in a mixture of *Buffer solution* and methanol (96:4) to obtain a solution having known concentrations of about 0.16 mg per mL and 0.08 mg per mL, respectively.

[Note—Use this solution within 10 minutes.]

Assay preparation—Weigh the contents of not fewer than 10 Capsules, mix, and transfer an accurately weighed portion of the powder, equivalent to about 8 mg of isoniazid, to a 100-mL volumetric flask, and add about 90 mL of *Buffer solution.* Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Buffer solution* to volume, and mix.

[Note—Use this solution within 2 hours.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains 5-µm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–6	100→0	0→100	linear gradient
6–15	0	100	isocratic

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 2.6 and 1.0 for rifampin and isoniazid, respectively; the column efficiency is not less than 50,000 and not less than 6,000 theoretical plates for rifampin and isoniazid, respectively; the tailing factors are not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) and isoniazid ($C_6H_7N_3O$) in the portion of Capsules taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Rifampin RS, calculated on the dried basis, or of USP Isoniazid RS, as appropriate, in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the corresponding analytes obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Topic/Question	Contact	Expert Committee
RIFAMPIN AND ISONIAZID CAPSULES	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 30(2)

Current DocID: GUID-A710DEC0-2A70-4BEC-B0EC-9EA9808E103D_1_en-US

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