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Rifampin, Isoniazid, and Pyrazinamide Tablets

» Rifampin, Isoniazid, and Pyrazinamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), and pyrazinamide ($C_5H_5N_3O$).

Packaging and storage—Preserve in tight, light-resistant containers at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Isoniazid RS
USP Pyrazinamide RS
USP Rifampin RS

Identification—

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

Test solution—Transfer an accurately weighed portion of ground Tablets, 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. Dissolve a quantity of USP Pyrazinamide RS in methanol to obtain a solution containing 15 mg per mL. Add an equal volume of acetone, and mix.

Application volume: 2 µL.

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

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

Dissolution ⟨711⟩—

Medium: simulated gastric fluid TS, without pepsin; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Standard stock solution—Prepare a solution in *Medium* having known concentrations of about 0.22 mg of USP Isoniazid RS and 1.3 mg of USP Pyrazinamide RS per mL. Use this solution on the day prepared.

Intermediate standard solution—Transfer about 27 mg of USP Rifampin RS, accurately weighed, to a 200-mL volumetric flask, add 50.0 mL of the *Standard stock solution,* and swirl to dissolve. Dilute with *Medium* to volume, and mix. Place this flask into the dissolution bath immediately prior to starting the tablet dissolution. Withdraw the flask from the dissolution bath at the same time that the solutions under test are withdrawn.

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

Standard solution—Transfer 10.0 mL of the *Intermediate standard solution* to a 50-mL volumetric flask, dilute with *Medium* to volume, and mix.

Procedure—Transfer 10.0 mL of the filtered solution under test to a separate 50-mL volumetric flask, dilute with *Medium* to volume, and mix. Concomitantly determine the UV absorbances at 475 nm of the solution obtained and the *Standard solution,* using the *Medium* as the blank. Calculate the quantity, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$) dissolved by the formula:

$$4500C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Rifampin RS in the *Standard solution;* and A_U and A_S are the absorbances of the solution under test and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of rifampin ($C_{43}H_{58}N_4O_{12}$) is dissolved in 30 minutes.

Determine the amount of $C_6H_7N_3O$ and $C_5H_5N_3O$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, 1 M monobasic potassium phosphate, and acetonitrile (860:100:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution of isonicotinic acid in *Medium* containing about 0.125 mg per mL. Transfer 10 mL of this solution and 4 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix.

Standard solution—Transfer 15.0 mL of the *Intermediate standard solution* to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix. This solution may be used for 20 hours.

Test solution—Withdraw 60 mL of the solution under test, and filter, discarding the first 20 mL of the filtrate. Centrifuge the filtrate for 5 minutes. Transfer 15.0 mL of this solution to a 100-mL volumetric flask containing 15 mL of 1 M dibasic potassium phosphate and 30 mL of *Mobile phase.* Dilute with *Mobile phase* to volume, and mix. This solution may be used for 20 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L44. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for isonicotinic acid, 1.0 for pyrazinamide, and 1.8 for isoniazid; and the resolution, *R,* between isonicotinic acid and pyrazinamide is not less than 2.5 and between pyrazinamide and isoniazid not less than 4.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviations determined from the pyrazinamide and isoniazid responses for replicate injections are not more than 1.5%.

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 areas for the major peaks. Calculate the quantity, in mg, of isoniazid ($C_6H_7N_3O$) dissolved by the formula:

$$6000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isoniazid RS in the *Standard solution;* and r_U and r_S are the isoniazid peak areas obtained from the *Test solution* and the *Standard solution,* respectively. Calculate the quantity, in mg, of pyrazinamide ($C_5H_5N_3O$) dissolved by the same formula, except to read "USP Pyrazinamide RS" where "USP Isoniazid RS" is specified, and "pyrazinamide" where "isoniazid" is specified.

Tolerances—Not less than 80% *(Q)* of the labeled amount of isoniazid ($C_6H_7N_3O$) and not less than 75% of the labeled amount of pyrazinamide ($C_5H_5N_3O$) are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Loss on drying—Dry about 100 mg of powdered Tablets, accurately weighed, 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, isoniazid, and pyrazinamide—

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, USP Isoniazid RS, and USP Pyrazinamide 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, 0.08 mg per mL, and 0.43 mg per mL, respectively.

[Note—Use this solution within 10 minutes.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity 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

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
6–15	0	100	isocratic

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.8, 0.7, and 1.0 for rifampin, isoniazid, and pyrazinamide, respectively; the resolution, *R,* between isoniazid and pyrazinamide is not less than 4; the column efficiency is not less than 50,000, not less than 6,000, and not less than 10,000 theoretical plates for rifampin, isoniazid, and pyrazinamide, respectively; the tailing factor is 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 quantities, in mg, of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), and pyrazinamide ($C_5H_5N_3O$) in the portion of Tablets 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 or of USP Pyrazinamide 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.

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

Topic/Question	Contact	Expert Committee
RIFAMPIN, ISONIAZID, AND PYRAZINAMIDE TABLETS	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

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