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Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride Tablets

» Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride 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 ($C_6H_7N_3O$), pyrazinamide ($C_5H_5N_3O$), and ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

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

USP Reference standards ⟨11⟩—
USP Ethambutol Hydrochloride RS
USP Isoniazid RS
USP Pyrazinamide RS
USP Rifampin RS

Identification—

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

B: The retention time of the ethambutol peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for ethambutol hydrochloride.*

Dissolution ⟨711⟩—

Medium: 10 mM pH 6.8 sodium phosphate buffer, prepared by dissolving 7 g of anhydrous dibasic sodium phosphate in 5 L of water, and adjusting with phosphoric acid to a pH of 6.8; 900 mL.

Apparatus 2: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amounts of rifampin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$), pyrazinamide ($C_5H_5N_3O$), and ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) dissolved using filtered portions of the solution under test and by employing the procedures set forth in the *Assay for rifampin, isoniazid, and pyrazinamide* and the *Assay for ethambutol hydrochloride.*

Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$, $C_5H_5N_3O$, and $C_{10}H_{24}N_2O_2 \cdot 2HCl$ is dissolved in 45 minutes.

Loss on drying—Dry about 100 mg of powdered Tablets 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 anhydrous 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 a 5-µm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as

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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 for rifampin, isoniazid, and pyrazinamide are about 1.8, 0.7, and 1.0, respectively; the resolution, *R,* between isoniazid and pyrazinamide is not less than 4; the column efficiencies, determined from the rifampin, isoniazid, and pyrazinamide peaks are not less than 50,000 theoretical plates, 6000 theoretical plates, and 10,000 theoretical plates, 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 the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for ethambutol hydrochloride—

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

Buffer solution—Mix 1.0 mL of triethylamine and 1 L of water, and adjust with phosphoric acid to a pH of 7.0.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ethambutol Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 30 mg of ethambutol hydrochloride, to a 100-mL volumetric flask, and add about 90 mL of *Diluent.* Sonicate for about 10 minutes, allow to equilibrate to room temperature, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter, discarding the first 10 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 15-cm column that contains a 5-µm base-deactivated packing L10. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 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 ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) 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 Ethambutol Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the ethambutol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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Topic/Question	Contact	Expert Committee
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