# Rifampicin, isoniazid and ethambutol hydrochloride tablets (Rifampicini, isoniazidi et ethambutoli hydrochloridi compressi)

**Category.** Antituberculosis drugs.

**Storage.** Rifampicin, Isoniazid and Ethambutol hydrochloride tablets should be kept in a tightly closed container, protected from light.

**Additional information.** Strength in the current WHO Model list of essential medicines: 150 mg Rifampicin, 75 mg Isoniazid and 275 mg Ethambutol hydrochloride.

The tablets are coated.

## Requirements

Comply with the monograph for "Tablets".

**Definition.** Rifampicin, Isoniazid and Ethambutol hydrochloride tablets contain Rifampicin, Isoniazid and Ethambutol hydrochloride. They contain not less than 90.0% and not more than 110.0% of the amounts of rifampicin ($C_{43}H_{58}N_4O_{12}$), isoniazid ($C_6H_7N_3O$) and ethambutol hydrochloride ($C_{10}H_{24}N_2O_2$, 2HCl) stated on the label.

**Manufacture.** The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the tablets. They ensure that, if tested, the tablets would comply with a loss on drying limit of not more than 30 mg/g when determined by drying freshly powdered tablets to constant mass under vacuum at 60°.

## Identity tests

• Either tests A and B or test C may be applied.

A. See the test described below under Assay method A. The retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the principal peaks in the chromatogram obtained with solution (2).

B. See the test described below under Assay method B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. Carry out test C.1 or, where UV detection is not available, test C.2.

> C.1 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 100 volumes of methanol R and 1.5 volumes of strong ammonia solution R as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions in methanol R. For solution (A) shake a quantity of the powdered tablets containing about 5 mg of Isoniazid for 15 minutes with 5 mL of methanol R, filter, and use the filtrate. For solution (B) use 1 mg of isoniazid RS and proportional quantities (according to the ratio in the tablet) of rifampicin RS, and ethambutol hydrochloride RS per mL of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, place in a chamber with iodine vapour, and allow to stand for 20 minutes. Examine the chromatogram immediately in ultraviolet light (254 nm).
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> The principal spots obtained with solution A correspond in position, appearance and intensity to those obtained with solution B.
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> C.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using the conditions described above under test C.1 butusing silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, place in a chamber with iodine vapour, and allow to stand for 20 minutes. Examine the chromatogram immediately in daylight.
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> The principal spots obtained with solution A correspond in position, appearance and intensity to those obtained with solution B.

**Dissolution test. [*To be added for rifampicin.]***

**Rifampicin-related substances.** Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, preparing the solutions and using the conditions given below under Assay method B.

Inject 20 µl each of solutions (1), (3) (4) and (5). The test is not valid unless in the chromatogram obtained with solution (4) the resolution between the peaks is at least 4.

In the chromatograms obtained with solutions (4) and (5) the following impurity peaks are eluted at the following relative retention with reference to rifampicin (retention time about 25 minutes): 3-(isonicotinoylhydrazinomethyl)rifamycin [the "hydrazone" resulting from reaction between 3-formylrifamycin and isoniazid] about 0.5; rifampicin quinone about 0.7.

In the chromatogram obtained with solution (1), the area of any peak corresponding to the hydrazone impurity is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (5.0%), the area of any peak corresponding to rifampicin quinone is not more than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (4.0%) and the area of any other peak is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (3) (1.5%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (3) (10.0% with reference to the content of rifampicin). Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%) and any peak with relative retention time less than 0.23 with reference to rifampicin.

**Assay**

A. ***For isoniazid and ethambutol hydrochloride***

Determine by *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups, (5 µm). As the mobile phase, use a solution prepared as follows: dissolve 50 g of ammonium acetate R and 0.2 g of copper(II) acetate R in 1000 mL of water R and adjust to pH 5.0 with glacial acetic acid R. Mix 940 mL of this solution with 60 mL of methanol R.

Prepare the following solutions in water. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powder containing about 100 mg of Ethambutol hydrochloride, accurately weighed, to a 500 mL volumetric flask. Dissolve in about 400 mL of water R by shaking for about 15 minutes. [If foaming occurs, use 400 mL of a 4% solution of methanol R in place of the water.] Dilute to 500 mL with water R. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtered solution. For solution (2) dissolve 27.3 mg of isoniazid RS and 100 mg of ethambutol hydrochloride RS in 500 mL of water R.

Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 270 nm.

Inject alternately 20 µl each of solutions (1) and (2). (The peak for isoniazid is eluted at a retention time of approximately 1.6 minutes, and that for ethambutol hydrochloride at a retention time of approximately 6 minutes.)

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of isoniazid, $C_6H_7N_3O$ and ethambutol hydrochloride, $C_{10}H_{24}N_2O_2$, 2HCl.

B. ***For rifampicin***

Prepare fresh solutions and perform the assay without delay. Low-actinic glassware is recommended.

Determine by *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups, (5 µm). As the mobile phase, use a mixture of 6 volumes of methanol R and 4 volumes of phosphate buffer pH 7.0 (potassium dihydrogen phosphate R (0.01 mol/l), adjusted with sodium hydroxide (0.1 mol/l)VS).

Prepare the following solutions in a mixture of 4 volumes of methanol R and 6 volumes of phosphate buffer pH 7.0. For solution (1) weigh and powder 20 tablets. Without delay, shake a quantity of the powder containing about 40 mg of Rifampicin in 200 mL and filter. Solution (2) contains 0.20 mg of rifampicin RS per mL. For solution (3) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10 µg of Rifampicin per mL. Solution (4) contains 0.2 mg of rifampicin RS per mL and 0.2 mg of rifampicin quinone RS per mL. For solution (5) dissolve 4 mg of rifampicin RS and 2 mg of isoniazid RS in 25.0 mL of acetic acid (~60g/l) TS and keep the solution at room temperature for 30 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Inject 20 µl of solution (4). The assay is not valid unless the resolution between the peaks is at least 4.

Inject alternately 20 µl each of solutions (1) and (2). Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of rifampicin, $C_{43}H_{58}N_4O_{12}$, in the tablets.