



WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

ISONIAZID AND ETHAMBUTOL HYDROCHLORIDE TABLETS

Final text for addition to *The International Pharmacopoeia*

This monograph was adopted at the Fortieth WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2005 for addition to the 4th edition of the International Pharmacopoeia.

Category. Antituberculosis drugs.

Storage. Isoniazid and Ethambutol hydrochloride tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 150 mg Isoniazid and 400 mg Ethambutol hydrochloride. Isoniazid and Ethambutol tablets are usually film-coated.

Requirements

Comply with the monograph for “Tablets”.

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

Identity tests

- Either test A or test B may be applied.
- A. See the Assay described below. The retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).
- B. Carry out test B.1. or, where UV detection is not available, test B.2.
 - B.1 Carry out the test as described under 1.14.1 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 μ l of each of the following two solutions in methanol R. For solution (A) shake a quantity of the powdered tablets equivalent to about 5 mg Isoniazid for 15 minutes with 5 ml of methanol R, filter, and use the filtrate. For solution (B) use 1 mg isoniazid RS and 2.67 mg 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 vapours, and allow to stand for 20 minutes. Examine the chromatogram immediately in ultraviolet light (254 nm).

The principal spots obtained with solution A correspond in position, appearance and intensity to those obtained with solution B.

B.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 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 equivalent to about 5 mg Isoniazid for 15 minutes with 5 ml of methanol R, filter, and use the filtrate. For solution (B) use 1 mg isoniazid RS and 2.67 mg 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 vapours, and allow to stand for 20 minutes. Examine the chromatogram immediately in daylight.

The principal spots obtained with solution A correspond in position, appearance and intensity to those obtained with solution B.

Dissolution/Disintegration

- Either test A or test B may be applied

A. Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 500 ml of dissolution buffer, pH 6.8, TS and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool to room temperature and dilute 5 ml to 20 ml with water [solution (3)]. Determine the content of isoniazid, $C_6H_7N_3O$, and ethambutol hydrochloride, $C_{10}H_{24}N_2O_2 \cdot 2HCl$ as described below under Assay using solution (3) in place of solution (1).

For each of the six tablets tested, calculate the total amount of isoniazid, $C_6H_7N_3O$, and ethambutol hydrochloride, $C_{10}H_{24}N_2O_2 \cdot 2HCl$ in the medium from the results obtained. For both substances the average amount in solution is not less than 85% of the amount declared on the label. For either substance, if the amount of obtained for one of the six tablets is less than 80 %, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 85%.

B. Disintegration. Comply with 5.4 Disintegration test for tablets and capsules, operating the apparatus for 15 minutes. If the tablets do not comply, carry out test A above.

Assay Determine by 1.14.4 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)¹. As the mobile phase, use a solution prepared as follows: dissolve 50 g ammonium acetate R and 0.2 g copper(II) acetate R in 1000 ml of water and adjust to pH 5.0 with glacial acetic acid R. Mix 940 ml of this solution with 60 ml methanol R.

¹ Luna® is suitable

Prepare the following solutions in water. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 100 mg Ethambutol hydrochloride, accurately weighed, to a 500 ml volumetric flask. Dissolve in about 400 ml water 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. 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 100 mg ethambutol hydrochloride RS and 37.5 mg isoniazid RS in 500 ml water.

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, $C_{10}H_{24}N_2O_2, 2HCl$.
