



**RIFAMPICIN, ISONIAZID AND PYRAZINAMIDE
DISPERSIBLE TABLETS**
**Revised draft proposal for *The International Pharmacopoeia*
(September 2007)**

REVISED DRAFT FOR DISCUSSION

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/07.222
International Pharmacopoeia monograph on Rifampicin, Isoniazid and Pyrazinamide
dispersible tablets

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RIFAMPICIN, ISONIAZID AND PYRAZINAMIDE DISPERSIBLE TABLETS

Revised Draft proposal for *The International Pharmacopoeia* (September 2007)

Category. Antituberculosis drugs.

Storage. Rifampicin, Isoniazid and Pyrazinamide dispersible tablets should be kept in a tightly closed container, protected from light.

Additional information. The following strengths are available as dispersible tablets:

60 mg Rifampicin, 30 mg Isoniazid and 150 mg Pyrazinamide.

Requirements

Comply with the monograph for "Tablets".

Definition. Rifampicin, Isoniazid and Pyrazinamide dispersible tablets contain Rifampicin, Isoniazid and Pyrazinamide in a suitable dispersible basis that may contain suitable flavouring agents. 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 pyrazinamide ($C_5H_5N_3O$) stated on the label.

Identity tests

- Either tests A and B or test C may be applied.
- A. See Assay method A described below. 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 Assay method B described below. 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 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 containing about 5 mg Isoniazid for 15 minutes with 5 ml, filter, and use the filtrate. For solution (B) use 1 mg isoniazid RS and proportional quantities (according to the ratio in the tablet) of rifampicin RS and pyrazinamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air and examine the chromatogram in ultraviolet light (254 nm).

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

- C.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using 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.

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

Disintegration. Carry out the test as described under 5.3 Disintegration test for tablets and capsules, but using water R at 15-25 °C. The tablets disintegrate within 3 minutes

Dissolution test. [To be added for rifampicin.]

Rifampicin-related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given below under Assay method B.

Inject alternately 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 greater 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 in the chromatogram obtained with solution (3) (10.0%). 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 a relative retention less than 0.23 with reference to rifampicin.

Assay

- A. **For isoniazid and pyrazinamide.** 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 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 containing about 30 mg Isoniazid, 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 30 mg isoniazid RS and a proportional quantity (according to the ratio in the tablet) of pyrazinamide 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 240 nm.

Inject 20 µl of solution (2). The assay is not valid unless the resolution between the isoniazid and pyrazinamide peaks, eluting in this order, is at least 2.

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 isoniazid, C₆H₇N₃O, and pyrazinamide, C₅H₅N₃O, in the tablets.

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

Determine by 1.14.4 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)¹. 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 Rifampicin in 200 ml and filter. Solution (2) contains 0.20 mg rifampicin RS per ml. For solution (3) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10 µg Rifampicin per ml. Solution (4) contains 0.2 mg rifampicin RS per ml and 0.2 mg rifampicin quinone RS per ml. For solution (5) dissolve 4 mg of rifampicin RS and 2 mg of isoniazid RS in 25 ml of acetic acid (~60 g/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, C₄₃H₅₈N₄O₁₂, in the tablets.

¹ Luna® is suitable.