World Health Organization

ISONIAZID TABLETS

(ISONIAZIDI COMPRESSI)

Draft proposal for revision in *The International Pharmacopoeia*

(May 2022)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (email: schmidth@who.int), with a copy to Ms Sinéad Jones (email: jonessi@who.int) by **15 July 2022**.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the "*Working documents in public consultation*" link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.893:

ISONIAZID TABLETS

(ISONIAZIDI COMPRESSI)

Description	Date
Proposal drafted.	June 2021
Laboratory investigations to verify the analytical provisions	June 2021 – March 2022
Presentation to the 57th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	April 2022
Draft proposal to be sent out for public consultation.	May – July 2022
Further follow-up action as required.	

***[Note from the Secretariat**. It is proposed to revise the monograph on Isoniazid Tablets in* The International Pharmacopoeia. *The monograph is based on laboratory investigations and information found in other pharmacopoeias or submitted by manufacturers.*

Changes to the current chapter are indicated in the text by <u>insert</u> or ~~delete~~.]

ISONIAZID TABLETS (ISONIAZIDI COMPRESSI)

Category. Antituberculosis medicine.

Storage. Isoniazid tablets should be kept in a well-closed container, protected from moisture and light.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): tablets: 100 mg to 300 mg; scored tablets: 50 mg. Strength in the current EML for Children: tablets: 100 mg to 300 mg; scored tablets 50 mg.

Requirements

The tablets comply with the monograph on *Tablets*.

Definition. Isoniazid tablets contain not less than 90.0% and not more than 110.0% of the amount of $C_6H_7N_3O$ stated on the label.

Identity tests

- Either test A alone or any two of tests B, C or D may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid, add 10 mL of ethanol (~750g/L) TS and shake for 15 minutes. Centrifuge and decant the supernatant liquid. Extract the remaining liquid with two further 10-mL quantities of ethanol (~750g/L) TS and evaporate the combined extracts to dryness. Carry out the examination with the residue as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS or with the *reference spectrum* of isoniazid.

B. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions and solutions given under "Assay". The

retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time and of the peak due to isoniazid in the chromatogram obtained with solution (2).

C. 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 5 volumes of ethyl acetate R, 2 volumes of acetone R, 2 volumes of methanol R, and 1 volume of water R as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (A), shake a quantity of the powdered tablets, nominally containing 0.1 g of Isoniazid with 10 mL of methanol R, filter, and use the filtrate. For solution (B), use a solution containing 10 mg of isoniazid RS per mL of methanol R. Develop the plate. After removing it from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm).

The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to isoniazid in the chromatogram obtained with solution (B).

C. Suspend a quantity of the powdered tablets, nominally equivalent to 0.1 g of Isoniazid, in 2 mL of water R and add 10 mL of a warm solution of vanillin (10 g/L) TS, allow to stand and scratch the wall of the test-tube with a glass rod; a yellowish precipitate is obtained. Filter, recrystallize from 5 mL of ethanol (~600 g/L) TS, and dry at 105 °C. The melting temperature is between 226 °C and 231 °C.

Dissolution. Carry out the test described under *5.5 Dissolution test for oral dosage forms*, using as the dissolution medium 900 mL of hydrochloric acid (~0.365 g/L) TS and rotating the paddle at 100 revolutions per minute. At 45 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature.

If necessary, dilute a suitable volume of the filtrate with dissolution medium to obtain a solution containing 0.055 mg of isoniazid per mL.

Measure the absorbance as described under *1.6 Spectrophotometry in the visible and ultraviolet regions* of the resulting solution in a cuvette with an optical pathlength of 10 mm maximum at about 266 nm, using the dissolution buffer as the blank.

For each of the tablets tested, calculate the total amount of Isoniazid ($C_6H_7N_3O$) in the medium using the absorptivity value of 44.0 for isoniazid ($A_{1cm}^{1\%}$=440). Evaluate the results as described under *5.5 Dissolution test for oral dosage forms*, Acceptance criteria. The amount of Isoniazid released is not less than 80 % (Q) of the amount declared on the label.

*[**Note from the Secretariat.** The absorptivity value of isoniazid will be verified during the establishment of isoniazid ICRS.]*

Impurity E (hydrazine). Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using a stainless steel column (25 cm x 4.6 mm) packed with end-capped 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 water R and acetonitrile R (40:60 v/v). Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 300 nm.

Prepare as a diluent a mixture of 50 volumes of water R and 50 volumes of acetonitrile R.

Prepare the following solutions freshly:

[1] An Inertsil ODS-3V or a Symmetry C18 column were found suitable.

For solution (A), dilute 1 mL of benzaldehyde R to 50 mL with methanol R. Use this solution within 4 hours.

For solution (1), dissolve a quantity of the powdered tablets, nominally containing 50.0 mg of Isoniazid in 1 mL of water R and mix with 5 mL of solution (A). Mix and allow to stand for 45 minutes. Then dilute to 10.0 mL with the solvent solution.

For solution (2), dissolve 20.0 mg of hydrazine sulfate R (equivalent to 4.926 mg of hydrazine) in water and dilute to 50.0 mL with the same solvent. Dilute 2.5 mL of this solution to 100.0 mL with water R. Mix 1.0 mL of this solution and 2.5 mL of solution (A) and allow to stand for 45 minutes. Then dilute this solution to 25.0 mL with the solvent solution. Dilute 7.5 mL of this solution to 10.0 mL with the solvent solution.

For solution (3), mix 1.0 mL of water R and 2.5 mL of solution (A) and allow to stand for 45 minutes. Then dilute this solution to 25.0 mL with the solvent solution. Dilute 7.5 mL of this solution to 10.0 mL with the solvent solution.

Inject 10 µL each of solutions (2) and (3).

Use the chromatogram obtained with solutions (2) and (3) to identify the peak due to the reaction product of benzaldehyde and hydrazine, benzaldehyde azine (benzaldehyde azine is eluted at about 20 minutes). The test is not valid unless, in the chromatogram obtained with solution (2), the signal-to-noise ratio of the peak due to benzaldehyde azine is at least 10.

Inject 10 µL each of solutions (1) and (2) and record the chromatograms for about 1.5 times the retention time of benzaldehyde azine.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to benzaldehyde azine in not greater than the area of the peak due to benzaldehyde azine in the chromatogram obtained with solution (2) (15 ppm).

Related substances. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated and end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).[2]

Use the following conditions for gradient elution:

- mobile phase A: 3 volumes of methanol R and 97 volumes of phosphate buffer pH 6.9.
- mobile phase B: methanol R.

Prepare the phosphate buffer pH 6.9 by dissolving 13.6 g of potassium dihydrogen phosphate R in 950 mL of water R, adjust the pH to 6.9 by adding sodium hydroxide (~420 g/L) TS, add 30 mg of triethanolamine R and dilute to 1000 mL with water R.

Time (minutes)	**Mobile phase A (% v/v)**	**Mobile phase B (% v/v)**	**Comments**
0–12	100	0	Isocratic
12–20	100 to 85	0 to 15	Linear gradient
20–28	85	15	Isocratic
28–29	85 to 100	15 to 0	Return to initial composition
29–40	100	0	Re-equilibration

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 266 nm.

Prepare the following solutions freshly using mobile phase A as a diluent:

[2] An Inertsil ODS-3V column was found suitable.

For solution (1), transfer a quantity of the powdered tablets, nominally containing 100 mg of Isoniazid, into a 100 mL volumetric flask. Add 40 mL of mobile phase A and sonicate for 10 minutes. Cool to room temperature, dilute to volume and filter. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. For solution (3), dissolve 5 mg of isonicotinic acid R (impurity A) 5 mg of isonicotinamide R (impurity B) and 5 mg of nicotinoyl hydrazide R (impurity D) and dilute to 50.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. Dilute 1.0 mL of this solution to 10.0 mL with solution (1).

Inject 10 µL each of solutions (1), (2) and (3).

Use the chromatogram obtained with solution (3) to identify the peak due to impurities A, B and D. The impurities are eluted, if present, at the following relative retention with reference to isoniazid (retention time about 9 minutes): impurity A about 0.40; impurity D about 1.2; impurity B about 1.4. The test is not valid unless, in the chromatogram obtained with solution (3), the peak-to-valley ratio (p/v) is at least 1.8, where Hp is the height above the baseline of the peak due to impurity D and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to isoniazid. Also, the test is not valid unless, in the chromatogram obtained with solution (2), the peak due to isoniazid is detected with a signal-to-noise ration of at least 10.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.4, is not greater than 2 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.5, is not greater than 2 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.2 %);

- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 1.4, is not greater than 2 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 1.4, is not greater than 2 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.2 %);
- the area of any other impurity peak is not greater than the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.2 %).
- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to impurities A, B, Cand D, is not greater than 10 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (1.0 %). Disregard any peaks with an area less than the area of the peak due to isoniazid in the chromatogram in the chromatogram obtained with solution (2) (0.1%).

Assay. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using the conditions given below under "Related substances" with the following modifications.

As the mobile phase, use a mixture of mobile phase A and methanol R (95:5 v/v). Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 266 nm.

Prepare the following solutions freshly in mobile phase A.

For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 60 mg of Isoniazid, into a 200 mL volumetric flask. Add 80 mL of mobile phase A and sonicate for 10 minutes. Cool to room temperature, dilute to volume with mobile phase and filter. For solution (2), dissolve 60 mg of Isoniazid RS and dilute to 200.0 mL with mobile phase A.

Inject 10 µL each of solutions (1) and (2) and record the chromatograms for about 2 times the retention time of isoniazid (isoniazid is eluted with a retention time of about 7 minutes.

Measure the areas of the peaks corresponding to isoniazid obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of Isoniazid ($C_6H_7N_3O$) in the tablets using the declared content of $C_6H_7N_3O$ in isoniazid RS.

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph on Isoniazid.

~~**Category.** Antituberculosis drug.~~

~~**Additional information.** Strength in the current WHO Model list of essential medicines: 100 - 300mg.~~

~~**Requirements**~~

~~Comply with the monograph for "Tablets".~~

~~Isoniazid tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_6H_7N_3O$ stated on the label.~~

~~**Identity tests**~~

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

~~A. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add 10ml of ethanol (~750g/l) TS and shake for 15 minutes. Centrifuge and decant the supernatant liquid. Extract the remaining liquid with two further 10 mL~~

quantities of ethanol (~750g/l) TS and evaporate the combined extracts to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS or with the *reference spectrum* of isoniazid.

B. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add 2.0ml of water, shake, and filter. Then add a mixture composed of 1.0ml of silver nitrate (40g/l) TS and 1.0ml of ammonia (~100g/l) TS; bubbles of nitrogen evolve, the mixture turns from yellow to black and a metallic silver mirror appears on the sides of the test tube.

C. To a quantity of the powdered tablets equivalent to about 1mg of Isoniazid add 50ml of ethanol (~750g/l) TS, shake, and filter. To 5ml of the filtrate add 0.1g of sodium tetraborate R and 5ml of 1-chloro-2,4-dinitrobenzene/ethanol TS, evaporate to dryness on a water-bath, and continue heating for a further 10 minutes. To the residue add 10ml of methanol R and mix; a reddish violet colour is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of ethyl acetate R, 2 volumes of acetone R, 2 volumes of methanol R, and 1 volume of water as the mobile phase. Apply separately to the plate 10 µl of each of the 3 following solutions. For solution (A), shake a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid with 10 mL of methanol R, filter, and use the filtrate. For solution (B), use 10 mg of isoniazid RS per mL of methanol R. For solution (C), dilute 1 volume of solution A to 100 volumes with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets equivalent to about 0.4 g of Isoniazid as completely as possible in water, filter, and wash the residue with sufficient water to produce 250 mL. Place 50 mL of the resulting solution in a titration vessel, add 50 mL of water, 20 mL of hydrochloric acid (~250g/L) TS, and 0.2g of potassium bromide R, and titrate with potassium bromate (0.0167 mol/L) VS as described under 2.7 Nitrite titration.

Each mL of potassium bromate (0.0167 mol/L) VS is equivalent to 3.429 mg of $C_6H_7N_3O$.

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. Measure the absorbance (1.6) of a 1 cm layer of the filtered sample, suitably diluted if necessary, at the maximum at about 263 nm. At the same time, measure the absorbance at the maximum at about 263 nm of a suitable solution of isoniazid RS in dissolution buffer, pH 6.8, TS, using the same buffer as the blank.

For each of the six tablets tested, calculate the total amount of isoniazid ($C_6H_7N_3O$) in the medium. The amount in solution for each tablet is not less than 80% of the amount declared on the label. If the amount 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 75% and the amount obtained for no tablet is less than 60%.

B. **Disintegration.** Comply with 5.3 Disintegration test for tablets and capsules, operating the apparatus for 10 minutes. If the tablets do not comply, carry out test A above.

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