World Health Organization

ISONIAZID

(ISONIAZIDUM)

Draft proposal for revision in *The International Pharmacopoeia*

(May 2022)

DRAFT FOR COMMENT

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/21.892:

ISONIAZID

(ISONIAZIDUM)

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 in* The International Pharmacopoeia. *The monograph is based on laboratory investigations and information found in other pharmacopoeias and in scientific literature.*

Comments are sought, in particular, on the suitability of identity test E (determination of the melting point of the reaction product of isoniazid and vanillin).

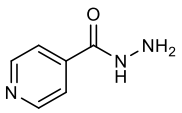
Changes to the current chapter are indicated in the text by insert or ~~delete~~.]

ISONIAZID (ISONIAZIDUM)

Molecular formula. $C_6H_7N_3O$

Relative molecular mass. 137.1

Graphic formula.



Chemical name. Pyridine-4-carbohydrazide; CAS Reg. No. 54-85-3.

Description. White or almost white, crystalline powder or colorless crystals.

Solubility. Freely soluble in water R, sparingly soluble in ethanol (~750 g/L) TS; practically insoluble in heptane R.

Category. Tuberculostatic.

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

Additional information. Isoniazid may exhibit polymorphism.

Requirements

Definition. Isoniazid contains not less than 99.0% and not more than 101.0% of $C_6H_7N_3O$, calculated with reference to the dried substance.

Identity tests

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

A. Carry out the examination 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.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and isoniazid RS in a small amount of ethanol (~750 g/L) TS and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS.

B. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Related substances" with the following modification. For solution (1), use a solution containing 0.1 mg of the test substance per mL of mobile phase A. For solution (2), use a solution containing 0.1 mg of isoniazid RS per mL of mobile phase A. Inject 10 µL of solutions (1) and (2). The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time and the UV spectrum of the peak due to isoniazid in the chromatogram obtained with solution (2).

C. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Related substances" with the following modification. For solution (1), use a solution containing 0.1 mg of the test substance per mL of mobile phase A. For solution (2), use a solution containing 0.1 mg of isoniazid RS per mL of mobile phase A. Inject 10 µL of solutions (1) and (2). The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to isoniazid in the chromatogram obtained with solution (2).

D. Carry out as described under *1.6 Spectrophotometry in the visible and ultraviolet regions*. Use a 0.01 mg per mL solution of the test substance in methanol R. The

adsorption spectrum of the test solution, when observed between 200 nm and 400 nm, exhibits a maximum at about 263 nm.

E. Dissolve 0.1 g of the test substance 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.

F. 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), dissolve 0.10 g of the test substance in 10 mL of methanol R. 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).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and color of solution. A solution of 0.50 g of the test substance in 10 mL of water R is clear and not more intensely colored than reference solution BY_7, when compared as described under *1.11.2 Degree of coloration of liquids*, Method II.

Sulfated ash (*2.3*). Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 0.05 g/mL solution of the test substance in carbon-dioxide-free water R, 6.0-8.0.

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:

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 50.0 mg of the test substance 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 diluent.

For solution (2), dissolve 20.0 mg of hydrazine sulfate R (equivalent to 4.925 mg of hydrazine) in water R 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

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

solution (A) and allow to stand for 45 minutes. Then dilute to 25.0 mL with the diluent. Dilute 7.5 mL of this solution to 10.0 mL with the diluent.

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 to 25.0 mL with the diluent. Dilute 7.5 mL of this solution to 10.0 mL with the diluent.

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:

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

- 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
29–35	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:

For solution (1), dissolve 25.0 mg of the test substance and dilute to 25.0 mL. 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), dilute 5.0 mL of solution (2) to 10.0 mL. For solution (4), 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 (3) and (4).

Use the chromatogram obtained with solution (4) to identify the peaks due to the 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, impurity F about 2.0; impurity C about 2.6. The test is not valid unless, in the chromatogram obtained with solution (4), 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 (3), the peak due to isoniazid is detected with a signal-to-noise ration of at least 10.

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

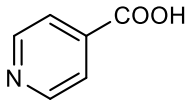
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.4, is not greater than 1.5 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.5, is not greater than 1.5 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 1.4, is not greater than the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.10 %);
- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 1.4, is not greater than the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.10 %);
- 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) (0.10 %).
- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to impurities A, B, C and D, is not greater than 5 times the

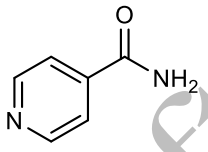
area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.5 %). Disregard any peaks with an area of less than 0.5 times the area of the peak due to isoniazid in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.250 g of the test substance in water R and dilute to 100.0 mL with the same solvent. To 20.0 mL of this solution, add 100 mL of water R, 20 mL of hydrochloric acid (~250 g/L) TS, 0.2 g of potassium bromide R, and 0.05 mL of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/L) VS, adding the titrant drop by drop and shaking till the red color disappears. Each mL of potassium bromate (0.0167 mol/L) VS is equivalent to 3.429 mg of $C_6H_7N_3O$.

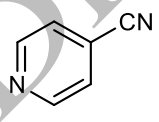
Impurities



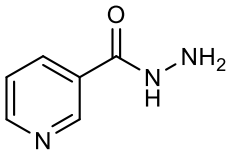
A. Pyridine-4-carboxylic acid (isonicotinic acid, isoniacin) (synthesis related impurity and degradation product).



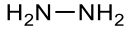
B. Pyridine-4-carboxamide (isonicotinamide) (synthesis related impurity).



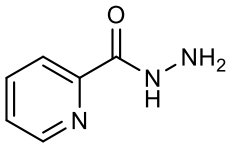
C. Pyridine-4-carbonitrile; 4-cyanopyridin (isonicotinonitrile) (synthesis related impurity).



D. Pyridine-3-carbohydrazide (nicotinoyl hydrazide) (synthesis related impurity).



E. Hydrazine (synthesis related impurity and degradation product).



F. Pyridine-2-carbohydrazide (Picolinohydrazide; 2-isoniazid)(synthesis related impurity)

Reagents to be established

Benzaldehyde R

C_7H_6O

Description. Colourless or slightly yellow liquid.

Solubility. Slightly soluble in water R, miscible with ethanol (~750 g/L) TS.

Relative density. d_{20}^{20} = about 1.05.

Distillation range. Not less than 95 per cent distils between 177 °C and 180 °C.

Storage. Protected from light.

Isonicotinic acid R

Pyridine-4-carboxylic acid, $C_6H_5NO_2$.

Description. Creamish-white powder.

Solubility. Sparingly soluble in water R.

Melting point. About 311 °C.

Isonicotinamide R

4-Pyridinecarboxamide, Pyridine-4-carboxamide, $C_6H_6N_2O$.

Description. White or almost white, crystalline powder.

Solubility. Soluble in water R.

Nicotinoyl hydrazide R

Pyridine-3-carbohydrazide, $C_6H_7N_3O$.

Description. White or almost white powder or crystalline powder.

Solubility. Soluble in water R.

Melting point. About 160 °C.

Triethanolamine R

$C_6H_{15}NO_3$

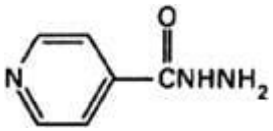
Description. Clear, viscous, colourless or slightly yellow liquid, very hygroscopic.

Relative density. d_{20}^{20} = 1.120 to 1.130.

~~**Molecular formula.** $C_6H_7N_3O$~~

~~**Relative molecular mass.** 137.1~~

Graphic formula.



Chemical name. 4-Pyridinecarboxylic acid hydrazide; CAS Reg. No. 54-85-3.

Other name. Isonicotinic acid hydrazide.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 8 parts of water and in 40 parts of ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Tuberculostatic.

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

Requirements

Definition. Isoniazid contains not less than 98.0% and not more than 101.0% of $C_6H_7N_3O$, calculated with reference to the dried substance.

Identity tests

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

A. Carry out the examination 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. Heat 0.05 g with about 1 g of anhydrous sodium carbonate R; pyridine, perceptible by its odour, is produced.

C. Dissolve 0.1 g in 2 mL of water and add 10 mL of a hot solution of vanillin (10 g/l) TS, scratch the inside of the test-tube and allow to stand; a yellow precipitate is obtained. Filter, recrystallize from 5 mL of ethanol (~600 g/l) TS, and dry at 105°C; melting temperature, about 227°C.

Melting range. 170-174°C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 μg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 mL of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105°C; it loses not more than 10 mg/g.

pH value. pH of a 0.05 g/mL solution in carbon-dioxide-free water R, 6.0-8.0.

Free hydrazine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 98 volumes of acetone R and 2 volumes of water as the mobile phase. Apply separately to the plate 10 μl of each of 2 solutions in a mixture of 1 volume of acetone R and 1 volume of water containing (A) 0.10 g of the test substance per mL, and (B) 20 μg of hydrazine hydrate R per mL. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, spray with 4-dimethylaminobenzaldehyde TS3, and examine the chromatogram in daylight. The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

Assay. Dissolve about 0.25 g, accurately weighed, in sufficient water to produce 100 mL. To 25.0 mL of this solution add 100 mL of water, 20 mL of hydrochloric acid (~250 g/l) TS, 0.2 g of potassium bromide R, and 3 drops of methyl red/ethanol TS.

~~Titrate with potassium bromate (0.0167 mol/l) VS, adding the titrant drop by drop and shaking till the red colour disappears. Repeat the operation without the substance being examined and make any necessary corrections. Each mL of potassium bromate (0.0167 mol/l) VS is equivalent to 3.429 mg of $C_6H_7N_3O$.~~

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