

## 2 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: <a href="mailto:schmidth@who.int">schmidth@who.int</a>), with a copy to Ms Sinéad Jones (email: <a href="mailto:jonessi@who.int">jonessi@who.int</a>) by **15 July 2022.** 

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (<a href="https://www.who.int/teams/health-product-and-policy-standards/standards-and-">https://www.who.int/teams/health-product-and-policy-standards/standards-and-</a>

<u>specifications/pharmaceuticals/current-projects</u>) 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 <u>jonessi@who.int</u> and your name will be added to our electronic mailing list.

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## SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.892:

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38 ISONIAZID

39 (ISONIAZIDUM)

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Date
June 2021
June 2021 – March 2022
April 2022
May – July 2022

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- 42 [Note from the Secretariat. It is proposed to revise the monograph on Isoniazid in The
- 43 International Pharmacopoeia. *The monograph is based on laboratory investigations and*
- 44 information found in other pharmacopoeias and in scientific literature.
- 45 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).
- 47 Changes to the current chapter are indicated in the text by <u>insert</u> or <del>delete</del>.]

#### **ISONIAZID (ISONIAZIDUM)**

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- 51 **Molecular formula.** C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O
- **Relative molecular mass.** 137.1
- 53 **Graphic formula.**

54 <sup>N</sup>

- 55 Chemical name. Pyridine-4-carbohydrazide; CAS Reg. No. 54-85-3.
- Description. White or almost white, crystalline powder or colorless crystals.
- 57 **Solubility.** Freely soluble in water R, sparingly soluble in ethanol (~750 g/L) TS;
- practically insoluble in heptane R.
- 59 **Category.** Tuberculostatic.
- **Storage.** Isoniazid should be kept in a well-closed container, protected from light.
- 61 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.
- 65 **Identity tests**
- Either test A or test B alone or any two of tests C, D, E or F may be applied.

67	<u>A.</u>	Carry out the examination as described under 1.7 Spectrophotometry in the					
68		infrared region. The infrared absorption spectrum is concordant with the					
69		spectrum obtained from isoniazid RS or with the reference spectrum of isoniazid.					
70		If the spectra thus obtained are not concordant, repeat the test using the residues					
71		obtained by separately dissolving the test substance and isoniazid RS in a small					
72		amount of ethanol (~750 g/L) TS and evaporating to dryness. The infrared					
73		absorption spectrum is concordant with the spectrum obtained from isoniazid RS.					
74	B.	Carry out the test as described under 1.14.4 High-performance liquid					
	<u>D.</u>						
75		chromatography using the conditions given under "Related substances" with the					
76		following modification. For solution (1), use a solution containing 0.1 mg of the					
77		test substance per mL of mobile phase A. For solution (2), use a solution					
78		containing 0.1 mg of isoniazid RS per mL of mobile phase A. Inject 10 µL of					
79		solutions (1) and (2). The retention time and the UV spectrum of the principal					
80		peak in the chromatogram obtained with solution (1) corresponds to the retention					
81		time and the UV spectrum of the peak due to isoniazid in the chromatogram					
82		obtained with solution (2).					
	<b>a</b>						
83	<u>C.</u>	Carry out the test as described under 1.14.4 High-performance liquid					
84		chromatography using the conditions given under "Related substances" with the					
85		following modification. For solution (1), use a solution containing 0.1 mg of the					
86		test substance per mL of mobile phase A. For solution (2), use a solution					
87		containing 0.1 mg of isoniazid RS per mL of mobile phase A. Inject 10 µL of					
88		solutions (1) and (2). The retention time of the principal peak in the					
89		chromatogram obtained with solution (1) corresponds to the retention time of the					
90		peak due to isoniazid in the chromatogram obtained with solution (2).					
91	D.	Carry out as described under 1.6 Spectrophotometry in the visible and ultraviolet					
92		regions. Use a 0.01 mg per mL solution of the test substance in methanol R. The					

93		adsorption spectrum of the test solution, when observed between 200 nm and			
94		400 nm, exhibits a maximum at about 263 nm.			
95	<u>E.</u>	Dissolve 0.1 g of the test substance in 2 mL of water R and add 10 mL of a warr			
96		solution of vanillin (10 g/L) TS, allow to stand and scratch the wall of the test-			
97		tube with a glass rod; a yellowish precipitate is obtained. Filter, recrystallize			
98		from 5 mL of ethanol (~600 g/L) TS, and dry at 105 °C. The melting temperature			
99		is between 226 °C and 231 °C.			
100	<u>F.</u>	Carry out the test as described under 1.14.1 Thin-layer chromatography, using			
101		silica gel R6 as the coating substance and a mixture of 5 volumes of ethyl acetate			
102	R, 2 volumes of acetone R, 2 volumes of methanol R, and 1 volume of water I				
103	as the mobile phase. Apply separately to the plate 10 µL of each of the following				
104		solutions. For solution (A), dissolve 0.10 g of the test substance in 10 mL of			
105	methanol R. For solution (B), use a solution containing 10 mg of isoniazid RS				
106	per mL of methanol R. Develop the plate. After removing it from the				
107		chromatographic chamber, allow it to dry in air and examine the chromatogram			
108		in ultraviolet light (254 nm).			
109	The principal spot in the chromatogram obtained with solution (A) corresponds				
110	in position, appearance and intensity with the spot due to isoniazid in the				
111	chromatogram obtained with solution (B).				
	**				
112		y metals. Use 1.0 g for the preparation of the test solution as described under			
113					
114	accor	ding to Method A; not more than 20 μg/g.			
115	<u>Clari</u>	ty and color of solution. A solution of 0.50 g of the test substance in 10 mL of			
116	water	R is clear and not more intensely colored than reference solution BY <sub>7</sub> , when			
117	compared as described under 1.11.2 Degree of coloration of liquids, Method II.				
118	<u>Sulfa</u>	ted 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
- 121 <u>R, 6.0-8.0.</u>
- 122 **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).<sup>1</sup>
- 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
- 130 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

<sup>&</sup>lt;sup>1</sup> 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. 140 Dilute 7.5 mL of this solution to 10.0 mL with the diluent. 141 For solution (3), mix 1.0 mL of water R and 2.5 mL of solution (A) and allow to stand 142 for 45 minutes. Then dilute to 25.0 mL with the diluent. Dilute 7.5 mL of this solution 143 to 10.0 mL with the diluent. 144 Inject 10 µL each of solutions (2) and (3). 145 Use the chromatogram obtained with solutions (2) and (3) to identify the peak due to 146 the reaction product of benzaldehyde and hydrazine, benzaldehyde azine (benzaldehyde 147 azine is eluted at about 20 minutes). The test is not valid unless, in the chromatogram 148 obtained with solution (2), the signal-to-noise ratio of the peak due to benzaldehyde 149 azine is at least 10. 150 Inject 10 µL each of solutions (1) and (2) and record the chromatograms for about 1.5 151 times the retention time of benzaldehyde azine. 152 In the chromatogram obtained with solution (1): 153 the area of any peak corresponding to benzaldehyde azine in not greater than the 154 area of the peak due to benzaldehyde azine in the chromatogram obtained with 155 156 solution (2) (15 ppm). **Related substances.** Carry out the test as described under 1.14.4 High-performance 157 liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with 158 base-deactivated and end-capped particles of silica gel, the surface of which has been 159 modified with chemically-bonded octadecylsilyl groups (5 µm).<sup>2</sup> 160 Use the following conditions for gradient elution: 161

<sup>&</sup>lt;sup>2</sup> 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.

<u>Time</u>	Mobile phase A	Mobile phase B	Comments
(minutes)	(% v/v)	(% v/v)	
<u>0–12</u>	<u>100</u>	<u>0</u>	<u>Isocratic</u>
12–20	100 to 85	<u>0 to 15</u>	Linear gradient
20–28	<u>85</u>	<u>15</u>	<u>Isocratic</u>
<u>28–29</u>	<u>85 to 100</u>	15 to 0	Return to initial composition
<u>29–35</u>	<u>100</u>	<u>0</u>	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 with solution (1).
- Inject  $10 \mu L$  each of solutions (3) and (4).

- Use the chromatogram obtained with solution (4) to identify the peaks due to the 179 impurities A, B and D. The impurities are eluted, if present, at the following relative 180 retention with reference to isoniazid (retention time about 9 minutes): impurity A about 181 0.40; impurity D about 1.2; impurity B about 1.4, impurity F about 2.0; impurity C 182 about 2.6. The test is not valid unless, in the chromatogram obtained with solution (4), 183 the peak-to-valley ratio (p/v) is at least 1.8, where Hp is the height above the baseline 184 of the peak due to impurity D and Hv is the height above the baseline of the lowest point 185 of the curve separating this peak from the peak due to isoniazid. Also, the test is not 186 valid unless, in the chromatogram obtained with solution (3), the peak due to isoniazid 187 is detected with a signal-to-noise ration of at least 10. 188
- 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

207 <u>area of the peak due to isoniazid in the chromatogram obtained with solution (2)</u> 208 <u>(0.5 %)</u>. 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  $C_6H_7N_3O$ .

### <u>Impurities</u>

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

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

223 <u>C. Pyridine-4-carbonitrile; 4-cyanopyridin (isonicotinonitrile) (synthesis related</u>
224 <u>impurity).</u>

- 226 D. Pyridine-3-carbohydrazide (nicotinoyl hydrazide) (synthesis related impurity).
- 227 H<sub>2</sub>N-NH<sub>2</sub>
- E. Hydrazine (synthesis related impurity and degradation product).

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

## 232 Reagents to be established

# 233 Benzaldehyde R

234 C<sub>7</sub>H<sub>6</sub>O

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- 235 Description. Colourless or slightly yellow liquid.
- 236 Solubility. Slightly soluble in water R, miscible with ethanol (~750 g/L) TS.
- 237 *Relative density.*  $d_{20}^{20} = \text{about } 1.05.$
- 238 Distillation range. Not less than 95 per cent distils between 177 °C and 180 °C.
- 239 Storage. Protected from light.

#### Isonicotinic acid R

241 Pyridine-4-carboxylic acid, C<sub>6</sub>H<sub>5</sub>NO<sub>2</sub>.

Relative molecular mass. 137.1

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Description. Creamish-white powder. 242 Solubility. Sparingly soluble in water R. 243 Melting point. About 311 °C. 244 Isonicotinamide R 245 4-Pyridinecarboxamide, Pyridine-4-carboxamide, C<sub>6</sub>H<sub>6</sub>N<sub>2</sub>O. 246 Description. White or almost white, crystalline powder. 247 Solubility. Soluble in water R. 248 Nicotinoyl hydrazide R 249 Pyridine-3-carbohydrazide, C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O. 250 Description. White or almost white powder or crystalline powder. 251 Solubility. Soluble in water R. 252 Melting point. About 160 253 Triethanolamine R 254 255 Description. Clear, viscous, colourless or slightly yellow liquid, very hygroscopic. 256 *Relative density.*  $d_{20}^{20} = 1.120$  to 1.130. 257 258 Molecular formula. C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O 259

#### Graphic formula.

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- 263 Chemical name. 4-Pyridinecarboxylic acid hydrazide; CAS Reg. No. 54-85-3.
- 264 Other name. Isonicotinic acid hydrazide.
- 265 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
- 267 slightly soluble in ether R.
- 268 Category. Tuberculostatic.
- Storage. Isoniazid should be kept in a well-closed container, protected from light.
- 270 Requirements
- 271 **Definition.** Isoniazid contains not less than 98.0% and not more than 101.0% of
- 272 C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O, calculated with reference to the dried substance.
- 273 **Identity tests**
- Either test A alone or tests B and C may be applied.
- 275 A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared
- 276 region. The infrared absorption spectrum is concordant with the spectrum obtained from
- 277 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
- 279 by its odour, is produced.

- 280 C. Dissolve 0.1 g in 2 mL of water and add 10 mL of a hot solution of vanillin (10 g/l)
- 281 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
- 283 temperature, about 227°C.
- 284 **Melting range.** 170-174°C.
- Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3
- 286 Limit test for heavy metals, Procedure 1; determine the heavy metals content according
- 287 to Method A; not more than  $20 \mu g/g$ .
- 288 Clarity and colour of solution. A solution of 0.50 g in 10 mL of water is clear and
- 289 <del>colourless.</del>
- 290 **Sulfated ash.** Not more than 1.0 mg/g.
- 291 Loss on drying. Dry to constant weight at 105°C; it loses not more than 10 mg/g.
- 292 **pH value.** pH of a 0.05 g/mL solution in carbon-dioxide-free water R, 6.0-8.0.
- 293 Free hydrazine. Carry out the test as described under 1.14.1 Thin layer
- 294 <u>chromatography</u>, using silica gel R1 as the coating substance and a mixture of 98
- 295 volumes of acetone R and 2 volumes of water as the mobile phase. Apply separately to
- 296 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
- 298 hydrate R per mL. After removing the plate from the chromatographic chamber, allow
- 299 it to dry in a current of air, spray with 4-dimethylaminobenzaldehyde TS3, and examine
- 300 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.
- 302 Assay. Dissolve about 0.25 g, accurately weighed, in sufficient water to produce 100
- 303 mL. To 25.0 mL of this solution add 100 mL of water, 20 mL of hydrochloric acid
- 304 (~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<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O.

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