



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**.

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35 SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.893:

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ISONIAZID TABLETS

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(ISONIAZIDI COMPRESSI)

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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.	

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

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

46

47 **ISONIAZID TABLETS (ISONIAZIDI COMPRESSI)**

48 **Category.** Antituberculosis medicine.

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

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

54 **Requirements**

55 The tablets comply with the monograph on [Tablets](#).

56 **Definition.** Isoniazid tablets contain not less than 90.0% and not more than 110.0% of
57 the amount of C₆H₇N₃O stated on the label.

58 **Identity tests**

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

60 A. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid, add
61 10 mL of ethanol (~750g/L) TS and shake for 15 minutes. Centrifuge and decant
62 the supernatant liquid. Extract the remaining liquid with two further 10-mL
63 quantities of ethanol (~750g/L) TS and evaporate the combined extracts to
64 dryness. Carry out the examination with the residue as described under 1.7
65 *Spectrophotometry in the infrared region*. The infrared absorption spectrum is
66 concordant with the spectrum obtained from isoniazid RS or with the *reference*
67 *spectrum* of isoniazid.

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

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

73 C. Carry out the test as described under 1.14.1 Thin-layer chromatography, using
74 silica gel R6 as the coating substance and a mixture of 5 volumes of ethyl acetate
75 R, 2 volumes of acetone R, 2 volumes of methanol R, and 1 volume of water R
76 as the mobile phase. Apply separately to the plate 10 µL of each of the following
77 solutions. For solution (A), shake a quantity of the powdered tablets, nominally
78 containing 0.1 g of Isoniazid with 10 mL of methanol R, filter, and use the filtrate.
79 For solution (B), use a solution containing 10 mg of isoniazid RS per mL of
80 methanol R. Develop the plate. After removing it from the chromatographic
81 chamber, allow it to dry in air and examine the chromatogram in ultraviolet light
82 (254 nm).

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

86 C. Suspend a quantity of the powdered tablets, nominally equivalent to 0.1 g of
87 Isoniazid, in 2 mL of water R and add 10 mL of a warm solution of vanillin (10
88 g/L) TS, allow to stand and scratch the wall of the test-tube with a glass rod; a
89 yellowish precipitate is obtained. Filter, recrystallize from 5 mL of ethanol
90 (~600 g/L) TS, and dry at 105 °C. The melting temperature is between 226 °C
91 and 231 °C.

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

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

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

102 For each of the tablets tested, calculate the total amount of Isoniazid (C₆H₇N₃O) in the
103 medium using the absorptivity value of 44.0 for isoniazid ($A_{1\text{cm}}^{1\%}=440$). Evaluate the
104 results as described under 5.5 Dissolution test for oral dosage forms, Acceptance
105 criteria. The amount of Isoniazid released is not less than 80 % (Q) of the amount
106 declared on the label.

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

109 **Impurity E (hydrazine).** Carry out the test as described under 1.14.4 High-
110 performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm)
111 packed with end-capped particles of silica gel, the surface of which has been modified
112 with chemically-bonded octadecylsilyl groups (5 µm).¹

113 As the mobile phase use a mixture of water R and acetonitrile R (40:60 v/v). Operate
114 with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet
115 spectrophotometer set at a wavelength of 300 nm.

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

118 Prepare the following solutions freshly:

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

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

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

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

129 For solution (3), mix 1.0 mL of water R and 2.5 mL of solution (A) and allow to stand
130 for 45 minutes. Then dilute this solution to 25.0 mL with the solvent solution. Dilute
131 7.5 mL of this solution to 10.0 mL with the solvent solution.

132 Inject 10 μ L each of solutions (2) and (3).

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

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

140 In the chromatogram obtained with solution (1):

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

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

148 Use the following conditions for gradient elution:

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

152 Prepare the phosphate buffer pH 6.9 by dissolving 13.6 g of potassium dihydrogen
153 phosphate R in 950 mL of water R, adjust the pH to 6.9 by adding sodium hydroxide
154 (~420 g/L) TS, add 30 mg of triethanolamine R and dilute to 1000 mL with water R.

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

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

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

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

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

166 Inject 10 μ L each of solutions (1), (2) and (3).

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

178 In the chromatogram obtained with solution (1):

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

- 185 • the area of any peak corresponding to impurity C, when multiplied by a
186 correction factor of 1.4, is not greater than 2 times the area of the peak due to
187 isoniazid in the chromatogram obtained with solution (2) (0.2 %);
- 188 • the area of any peak corresponding to impurity D, when multiplied by a
189 correction factor of 1.4, is not greater than 2 times the area of the peak due to
190 isoniazid in the chromatogram obtained with solution (2) (0.2 %);
- 191 • the area of any other impurity peak is not greater than the area of the peak due to
192 isoniazid in the chromatogram obtained with solution (2) (0.2 %).
- 193 • The sum of the areas of all impurity peaks, including the corrected areas of any
194 peaks corresponding to impurities A, B, C and D, is not greater than 10 times the
195 area of the peak due to isoniazid in the chromatogram obtained with solution (2)
196 (1.0 %). Disregard any peaks with an area less than the area of the peak due to
197 isoniazid in the chromatogram in the chromatogram obtained with solution (2)
198 (0.1%).

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

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

205 Prepare the following solutions freshly in mobile phase A.

206 For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered
207 tablets, nominally containing 60 mg of Isoniazid, into a 200 mL volumetric flask. Add
208 80 mL of mobile phase A and sonicate for 10 minutes. Cool to room temperature, dilute
209 to volume with mobile phase and filter. For solution (2), dissolve 60 mg of Isoniazid
210 RS and dilute to 200.0 mL with mobile phase A.

211 Inject 10 µL each of solutions (1) and (2) and record the chromatograms for about 2
212 times the retention time of isoniazid (isoniazid is eluted with a retention time of about
213 7 minutes.

214 Measure the areas of the peaks corresponding to isoniazid obtained in the
215 chromatograms of solutions (1) and (2) and calculate the percentage content of Isoniazid
216 (C₆H₇N₃O) in the tablets using the declared content of C₆H₇N₃O in isoniazid RS.

217 **Impurities**

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

220

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

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

224 **Requirements**

225 Comply with the monograph for "[Tablets](#)".

226 ~~Isoniazid tablets contain not less than **90.0%** and not more than **110.0%** of the amount~~
227 ~~of C₆H₇N₃O stated on the label.~~

228 **Identity tests**

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

230 ~~A. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add~~
231 ~~10ml of ethanol (~750g/l) TS and shake for 15 minutes. Centrifuge and decant~~
232 ~~the supernatant liquid. Extract the remaining liquid with two further 10 mL~~

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

238 B. — To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add
239 2.0ml of water, shake, and filter. Then add a mixture composed of 1.0ml of silver
240 nitrate (40g/l) TS and 1.0ml of ammonia (~100g/l) TS; bubbles of nitrogen
241 evolve, the mixture turns from yellow to black and a metallic silver mirror
242 appears on the sides of the test tube.

243 C. — To a quantity of the powdered tablets equivalent to about 1mg of Isoniazid add
244 50ml of ethanol (~750g/l) TS, shake, and filter. To 5ml of the filtrate add 0.1g
245 of sodium tetraborate R and 5ml of 1-chloro-2,4-dinitrobenzene/ethanol TS;
246 evaporate to dryness on a water bath, and continue heating for a further 10
247 minutes. To the residue add 10ml of methanol R and mix; a reddish-violet colour
248 is produced.

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

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

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

267 Each mL of potassium bromate (0.0167 mol/L) VS is equivalent to 3.429 mg of
268 $C_6H_7N_3O$.

269 **Dissolution/Disintegration**

270 • ~~Either test A or test B may be applied~~

271 A. ~~Dissolution. Carry out the test as described under [5.5 Dissolution test for solid](#)~~
272 ~~[oral dosage forms](#), using as the dissolution medium, 500 mL of dissolution buffer,~~
273 ~~pH 6.8, TS and rotating the paddle at 75 revolutions per minute. At 30 minutes,~~
274 ~~withdraw a sample of 10 mL of the medium through an in line filter. Measure~~
275 ~~the [absorbance \(1.6\)](#) of a 1 cm layer of the filtered sample, suitably diluted if~~
276 ~~necessary, at the maximum at about 263 nm. At the same time, measure the~~
277 ~~absorbance at the maximum at about 263 nm of a suitable solution of isoniazid~~
278 ~~RS in dissolution buffer, pH 6.8, TS, using the same buffer as the blank.~~

279 ~~For each of the six tablets tested, calculate the total amount of isoniazid~~
280 ~~($C_6H_7N_3O$) in the medium. The amount in solution for each tablet is not less~~
281 ~~than 80% of the amount declared on the label. If the amount obtained for one of~~
282 ~~the six tablets is less than 80%, repeat the test using a further six tablets; the~~
283 ~~average amount for all 12 tablets tested is not less than 75% and the amount~~
284 ~~obtained for no tablet is less than 60%.~~

285 ~~B. **Disintegration.** Comply with [5.3 Disintegration test for tablets and capsules](#),~~
286 ~~operating the apparatus for 10 minutes. If the tablets do not comply, carry out~~
287 ~~test A above.~~

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Draft for comments