



1 ETHANOL 96% (V/V)

2 (ETHANOLUM 96% (V/V))

3 Draft proposal for inclusion for *The International Pharmacopoeia*

4 (January 2021)

5 *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 (schmidth@who.int), with a copy to Ms Claire Vogel (vogelc@who.int) by **31 March 2021**.

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35 SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.872:
36
37 **ETHANOL 96% (V/V)**
38
39 **(ETHANOLUM 96% (V/V))**
40
41

Description	Date
Monograph drafted based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group.	December 2020
Draft monograph sent out for public consultation.	February – March 2021
Discussion at the Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines	May 2021
Presentation to the 56 th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2021
Further follow-up action as required.	

42
43
44 *[Note from the Secretariat. It is proposed to include the monograph on Ethanol 96% (V/V) in*
45 *The International Pharmacopoeia.*

46
47 *The monograph is based on the corresponding, internationally-harmonized text developed by*
48 *the Pharmacopoeial Discussion Group (PDG). Editorial modifications have been made in*
49 *order to be in line with the style used in The International Pharmacopoeia.]*

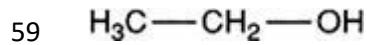
52 **ETHANOL 96% (V/V) (ETHANOLUM 96% (V/V))**

53

54 *This monograph is based on the corresponding, internationally-harmonized text developed by*
55 *the Pharmacopoeial Discussion Group (PDG). Editorial modifications have been made in*
56 *order to be in line with the style used in The International Pharmacopoeia.*

57

58 **Graphic formula.**



60

61 **Molecular formula.** $\text{C}_2\text{H}_6\text{O}$

62

63 **Relative molecular mass.** 46.07

64

65 **Chemical name.** Ethyl alcohol; ethanol; CAS Reg. No. 64-17-5.

66

67 **Other name.** Alcohol 96% (V/V).

68

69 **Description.** A colourless, clear and mobile liquid.

70

71 **Miscibility.** Miscible with water R.

72

73 **Category.** Solvent; antiseptic.

74

75 **Storage.** Ethanol 96% (V/V) should be kept in a well-closed container and stored, whenever
76 possible, at a temperature between 8 and 15 °C.

77

78 **Additional information.** Ethanol 96% (V/V) contains approximately 4% (V/V) of water. It is
79 flammable, burning with a blue smokeless flame, and hygroscopic. Boiling point, about 79 °C.

80

81

82

83

84

85 **Requirements**

86

87 **Definition.** Ethanol 96% (V/V) contains not less than 95.1% (V/V) and not more than 96.9%
88 (V/V) of C₂H₆O, corresponding to not less than 92.6% (m/m) and not more than 95.2% (m/m)
89 of C₂H₆O, at 20 °C.

90

91 **Identity tests**

92

93 A. Determine the relative density d_{20}^{20} of the test substance as described under *1.3*
94 *Determination of mass density, relative density and weight per millilitre*. The relative
95 density d_{20}^{20} is 0.805 to 0.812.

96

97 B. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The
98 infrared absorption spectrum of the test substance is concordant with the reference
99 spectrum of ethanol 96% (V/V)

100

101 **Clarity and colour of solution.** The test substance is clear when tested as described under
102 *1.18 Clarity and degree of opalescence of liquids [Note from the Secretariat. Chapter 1.18 is*
103 *currently under elaboration]* and colourless when compared with water R as described under
104 *1.11.2 Degree of coloration of liquids*, Method II. Dilute 1.0 mL to 20 mL with water R. After
105 standing for 5 minutes, the dilution remains clear when compared with water R.

106

107 **Non-volatile residue.** Place 100 mL of the test substance in a porcelain dish and heat on a
108 water-bath until volatilized, dry the residue at 105 °C for 1 hour, and weigh; not more than 2.5
109 mg (25 ppm m/V).

110

111 **Acidity or alkalinity.** Add 20 mL of carbon-dioxide-free water R and 0.1 mL of
112 phenolphthalein solution TS to 20 mL of the test substance; the solution is colourless. Add 0.1
113 mL of carbonate-free sodium hydroxide (0.01 mol/L) VS; the solution is pink (30 ppm,
114 expressed as acetic acid).

115

116 **Absorbance.** Record the absorption spectrum (1.6) of the test substance in a cuvette or cell
117 with an optical pathlength of 50 mm against water R between 235 nm and 340 nm. The

118 absorbance at 240 nm is not more than 0.40, not more than 0.30 between 250 nm and 260 nm,
119 and not more than 0.10 between 270 nm and 340 nm. The spectrum shows a steadily
120 descending curve with no observable peaks or shoulders.

121

122 **Volatile impurities.** Carry out the test as described under *1.14.5 Gas chromatography.*

123

124 Use a fused-silica column (30 m × 0.32 mm) coated with a stationary phase of
125 cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R (1.8 µm).

126

127 As a detector, use a flame ionization detector.

128

129 Use helium R as the carrier gas with a linear velocity of 35 cm/s. Use a split ratio of 1:20.

130

131 Maintain the temperature of the column at 40 °C for 12 minutes. Increase the temperature at a
132 rate of 10 °C per minute to 240 °C, and then maintain it at this temperature for 10 minutes.

133 Maintain the temperatures of the injection port and that of the detector at 200 °C and 280 °C,
134 respectively.

135

136 Prepare the following six solutions: For solution (1), use the test substance. For solution (2),
137 add 150 µL of 4-methylpentan-2-ol R to 500.0 mL of the test substance. For solution (3), dilute
138 100 µL of anhydrous methanol R to 50.0 mL with the test substance. Dilute 5.0 mL of the
139 solution to 50.0 mL with the test substance. For solution (4), dilute 50 µL of anhydrous
140 methanol R and 50 µL of acetaldehyde R to 50.0 mL with the test substance. Dilute 100 µL of
141 the solution to 10.0 mL with the test substance. For solution (5), dilute 150 µL of acetal R to
142 50.0 mL with the test substance. Dilute 100 µL of the solution to 10.0 mL with the test
143 substance. For solution (6), dilute 100 µL of benzene R to 100.0 mL with the test substance.
144 Dilute 100 µL of the solution to 50.0 mL with the test substance.

145

146 Inject alternately 1 µL each of solutions (1), (2), (3), (4), (5) and (6) and record the
147 chromatograms.

148

149 The test is not valid unless the resolution between the peaks corresponding to acetaldehyde (the
150 first peak) and methanol (the second peak) in the chromatogram obtained with solution (4) is
151 at least 1.5.

152

153 *Methanol (impurity F)*

154

155 In the chromatogram obtained with solution (1), the area of any peak corresponding to
156 methanol is not greater than 0.5 times the area of the peak due to methanol in the chromatogram
157 obtained with solution (3) (200 ppm (V/V)).

158

159 *Acetaldehyde (impurity B) and acetal (impurity A)*

160

161 Calculate the sum of the contents of acetaldehyde and acetal in parts per million (V/V) using
162 the following expression:

163

$$164 \frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E} \times \frac{44.05}{118.2}$$

165 where

A_E = area of any peak corresponding to acetaldehyde in the chromatogram obtained with solution (1);

A_T = area of the peak due to acetaldehyde in the chromatogram obtained with solution (4);

C_E = area of any peak corresponding to acetal in the chromatogram obtained with solution (1);

C_T = area of the peak due to acetal in the chromatogram obtained with reference solution (5);

44.05 = molecular mass of acetaldehyde;

118.2 = molecular mass of acetal.

166

167 The sum of the contents is not greater than 10 ppm (V/V), expressed as acetaldehyde.

168

169

170 *Benzene (impurity D)*

171

172 Calculate the content of benzene in parts per million (V/V) using the following expression:

173

$$\frac{2B_E}{B_T - B_E}$$

174 where

B_E = area of any peak corresponding to benzene in the chromatogram obtained with solution (1);

B_T = area of the peak due to benzene in the chromatogram obtained with solution (6).

175

176 The content of benzene is not greater than 2 ppm (V/V).

177

178 If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

180

181 *Other volatile impurities*

182

183 In the chromatogram obtained with solution (2), the sum of the areas of the peaks for any other 184 impurities is not greater than the area of the peak due to 4-methylpentan-2-ol (300 ppm). 185 Disregard any peak with an area less than 0.03 times than the area of the peak due to 4- 186 methylpentan-2-ol (9 ppm).

187

188 **Assay.** Calculate the mass density at 20 °C (ρ_{20}) of the test substance using the value for the 189 relative density d_{20}^{20} obtained in identity test A. Determine the % (V/V) of C_2H_6O using the 190 alcoholimetric table given in 1.3.2. *[Note from the Secretariat. Chapter 1.3.2 is currently 191 under revision. The revised version will include the mentioned alcoholimetric table]*

192

193 **Impurities**

194

195 *[Note from the Secretariat. The chemical structures will be added at a later stage.]*

196

197 A. 1,1-Diethoxyethane (acetal)

- 198 B. Acetaldehyde
- 199 C. Propan-2-one (acetone)
- 200 D. Benzene
- 201 E. Cyclohexane
- 202 F. Methanol
- 203 G. Butan-2-one (methyl ethyl ketone)
- 204 H. 4-Methylpentan-2-one (methyl isobutyl ketone)
- 205 I. Propan-1-ol (propanol)
- 206 J. Propan-2-ol (isopropyl alcohol)
- 207 K. Butan-1-ol (butanol)
- 208 L. Butan-2-ol
- 209 M. 2-Methylpropan-1-ol (isobutanol)
- 210 N. Furan-2-carbaldehyde (furfural)
- 211 O. 2-Methylpropan-2-ol (1,1-dimethylethyl alcohol)
- 212 P. 2-Methylbutan-2-ol
- 213 Q. Pentan-2-ol
- 214 R. Pentan-1-ol (pentanol)
- 215 S. Hexan-1-ol (hexanol)
- 216 T. Heptan-2-ol
- 217 U. Hexan-2-ol
- 218 V. Hexan-3-ol

219

220 **New reagents for Anhydrous Ethanol and Ethanol 96% (V/V) monographs**

221

222 **Phenolphthalein solution TS**

223

224 *Procedure.* Dissolve 0.1 g of phenolphthalein R in 80 mL of ethanol (~750 g/L) TS and dilute
225 to 100 mL with water R.

226

227 *Test for sensitivity.* Add 0.1 mL of the phenolphthalein solution to 100 mL of carbon dioxide-
228 free water R; the solution is colourless. Not more than 0.2 mL of sodium hydroxide (0.02
229 mol/L) VS is required to change the colour to pink.

230

231 *Colour change.* pH 8.2 (colourless) to pH 10.0 (red).

232

233 **Cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R**

234

235 Polysiloxane substituted with 3% cyanopropyl groups, 3% of phenyl groups and 94% of methyl
236 groups.

237

238 **4-Methylpentan-2-ol R**

239

240 4-Methyl-2-pentanol; C₆H₁₄O

241

242 *Description.* Clear, colourless, volatile liquid.

243

244 *Refractive index.* n_D^{20} = about 1.411

245

246 *Relative density.* d_4^{20} = about 0.802

247

248 *Boiling point.* About 132 °C.

249

250 **Methanol, anhydrous R**

251

252 *Procedure.* Treat 1000 mL of methanol R with 5 g of magnesium R. If necessary initiate the
253 reaction by adding 0.1 mL of mercuric chloride (54 g/L) TS. When the evolution of gas has
254 ceased, distil the liquid and collect the distillate in a dry container protected from moisture.

255

256 *Water (2.8).* Not more than 0.3 g/L.

257

258 **Magnesium R**

259

260 Mg

261

262 *Description.* Silver-white ribbon, turnings or wire, or a grey powder.

263

264 **Mercuric chloride (54 g/L) TS**

265

266 A solution of mercuric chloride R containing 54 g of HgCl₂ per litre.

267

268 **Acetal R**

269

270 Acetaldehyde diethyl acetal; 1,1-Diethoxyethane; C₆H₁₄O₂

271

272 *Description.* Clear, colourless, volatile liquid.

273

274 *Miscibility.* Miscible with water and with ethanol (~750 g/L) TS.

275

276 *Refractive index.* n_D^{20} = about 1.382

277

278 *Relative density.* d_{20}^{20} = about 0.824

279

280 *Boiling point.* About 103°C

281

282 **Amendment to existing reagent entry**

283

284 The existing entry for Benzene will be replaced with the following:

285

286 **Benzene**

287

288 C₆H₆ (SRIP, 1963, p.48)

289

290 Where benzene is used to prepare a reference solution, for safety reasons, the pure reagent may
291 be replaced by a commercially available reference material containing a certified amount of
292 benzene.

293

294
