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3 **REVISION OF THE MONOGRAPH ON**
4 **ETHINYLESTRADIOL**
5 **(ETHINYLESTRADIOLUM)**

6 **Draft proposal for inclusion in *The International Pharmacopoeia***
7 **(December 2018)**

8 ***DRAFT FOR COMMENTS***

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Please send any comments you may have on the attached text to Dr Herbert Schmidt, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (schmidth@who.int), with a copy to Ms Sinead Jones (jonessi@who.int) by **28 February 2019**.

Medicines Quality Assurance working documents will only be sent out electronically and will also be placed on the Medicines website for comment under “Current projects”. If you have not already received our draft working documents, please send your email address to jonessi@who.int and we will add your name to our electronic mailing list.

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55 SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/18.781:

56 **Draft proposal for inclusion in *The International Pharmacopoeia***

57 **REVISION OF THE MONOGRAPH ON**

58 **ETHINYLESTRADIOL**

59 **(ETHINYLESTRADIOLUM)**

60

Revision or the monograph prepared.	September 2018
Presentation to the WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2018
Draft revision sent out for public consultation	December 2018 – February 2019
Further follow-up action as required.	

61

62 *[Note from the Secretariat. It is proposed to revise the monograph on Ethinylestradiol as follows:*

63

- 64 • *Replace the existing TLC method to test for related substances with an HPLC method.*
- 65 • *Add an alternative assay method.*
- 66 • *Add an alternative identity test C by HPLC and revise the identity test B by TLC.*
- 67 • *Add a transparency list to the monograph.*

68

69 *The proposed changes are based on information found in the European Pharmacopoeia and in*
70 *Kommentar zum Europäischen Arzneibuch, Gesamtwerk mit 53. Aktualisierungslieferung 2016,*
71 *Wissenschaftliche Verlagsgesellschaft Stuttgart.*

72

73 *Changes from the current monograph are indicated in the text by insert or ~~delete~~.*

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Draft proposal for inclusion in *The International Pharmacopoeia*

REVISION OF THE MONOGRAPH ON

ETHINYLESTRADIOL

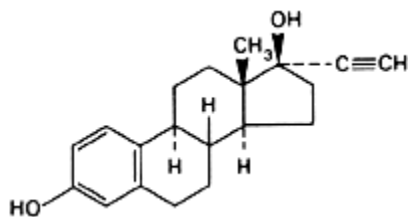
(ETHINYLESTRADIOLUM)

Ethinylestradiol (Ethinylestradiolum)

Molecular formula. $C_{20}H_{24}O_2$

Relative molecular mass. 296.4

Graphic formula.



Chemical name. 19-Nor-17 α -pregna-1,3,5(10)-trien-20-yne-3,17-diol; ~~17-ethynyl-estra-1,3,5,(10)-triene-3,17 β -diol~~; CAS Reg. No. 57-63-6.

Description. A white to slightly yellowish white, crystalline powder; ~~odourless~~.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS; soluble in acetone R, ~~and~~ dioxan R and dilute alkaline solutions.

Category. Estrogen.

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

Additional information. Ethinylestradiol may exhibit polymorphism. ~~may exist in 2 polymorphic forms one of which melts at about 183°C, the other, metastable, at about 143°C.~~

108 Requirements

109

110 **Definition.** Ethinylestradiol contains not less than 97.597.0% and not more than 102.0% of
111 $C_{20}H_{24}O_2$, calculated with reference to the dried substance.

112

113 Identity tests

114

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

116

117 A. Carry out the examination as described under [1.7 Spectrophotometry in the infrared region](#).
118 The infrared absorption spectrum is concordant with the spectrum obtained from
119 ethinylestradiol RS or with the reference spectrum of ethinylestradiol.

120

121 If the spectrum thus obtained are not concordant, repeat the test using the residues obtained
122 by separately dissolving the test substance and ethinylestradiol RS in a small amount of
123 methanol R and evaporating to dryness. The infrared absorption spectrum is concordant
124 with the spectrum obtained from ethinylestradiol RS. ~~If the spectrum obtained from the solid~~
125 state of the test substance is not concordant with the spectrum obtained from the reference
126 substance, compare the spectra of solutions in chloroform R containing 30 mg/mL, using a
127 path length of 0.2 mm.

128

129 B. Carry out the test as described under [1.14.1 Thin-layer chromatography](#) using silica gel R1
130 as the coating substance and a mixture of 10 volume of dehydrated ethanol R and 90
131 volumes of toluene R as the mobile phase. Apply separately to the plate 5 μ L of each of two
132 solutions in a mixture of 10 volumes of methanol R and 90 volumes of dichloromethane R
133 containing (A) 1.0 mg of the test substance per mL, and (B) 1.0 mg of ethinylestradiol RS
134 per mL. Develop the plate for a distance of 15 cm. After removing the plate from the
135 chromatographic chamber, allow it to air dry until the solvents have evaporated, heat at
136 110 °C for 10 minutes, spray the hot plate with sulfuric acid/ethanol (20%) TS and heat
137 again at 110 °C for 10 minutes. Allow to cool and examine the chromatogram in daylight
138 and in ultraviolet light (365 nm). The principal spot obtained with solution (A) corresponds
139 in position, appearance, and intensity with that obtained with solution (B). ~~Carry out the test~~
140 as described under [1.14.1 Thin-layer chromatography](#), using kieselguhr R1 as the coating

141 substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to
142 impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the
143 solvent has reached a height of at least 16 cm, remove the plate from the chromatographic
144 chamber and allow it to stand at room temperature until the solvent has completely
145 evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in
146 the same direction as the impregnation. Use toluene R as the mobile phase. Apply separately
147 to the plate 2 μ L of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1
148 volume of methanol R containing (A) 1.0 mg of the test substance per mL, and (B) 1.0 mg
149 of ethinylestradiol RS per mL. Develop the plate for a distance of 15 cm. After removing the
150 plate from the chromatographic chamber, allow it to dry in air until the solvents have
151 evaporated, heat at 120°C for 15 minutes, spray with 4-toluenesulfonic acid/ethanol TS, and
152 then heat at 120°C for 5–10 minutes. Allow to cool, and examine the chromatogram in
153 daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A
154 corresponds in position, appearance, and intensity with that obtained with solution B.

155
156 C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using
157 the conditions and solutions given under “Assay”, Method A. The retention time of the
158 principal peak in the chromatogram obtained with solution (1) corresponds to the retention
159 time of the peak due to ethinylestradiol in the chromatogram obtained with solution (5).

160
161 **Specific optical rotation.** Use a 4.0 mg/mL solution in pyridine R and calculate with reference to
162 the dried substance; $[\alpha]_{\text{D}}^{20^{\circ}\text{C}} = -27.0^{\circ}$ to -30.0° .

163
164 **Loss on drying.** Dry to constant weight at 105°C; it loses not more than 10 mg/g.

165
166 **Related substances.** Carry out the test as described under 1.14.4 High-performance liquid
167 chromatography, using a stainless steel column (25 cm \times 4.6 mm) packed with end-capped
168 particles of silica gel, the surface of which has been modified with chemically-bonded butylsilyl
169 groups (5 μ m).

170
171 Use the following conditions for gradient elution:

172
173 mobile phase A: 30 volumes of acetonitrile for chromatography R and 70 volumes of water R;

174
175 mobile phase B: 25 volumes of water R and 75 volumes of acetonitrile for chromatography R.

176
177

<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>0–35</u>	<u>100</u>	<u>0</u>	<u>Isocratic</u>
<u>35–65</u>	<u>100 to 0</u>	<u>0 to 100</u>	<u>Linear gradient</u>
<u>65–66</u>	<u>0 to 100</u>	<u>100 to 0</u>	<u>Return to initial</u> <u>composition</u>
<u>66–75</u>	<u>100</u>	<u>0</u>	<u>Re-equilibration</u>

178
179 Prepare the following solutions using a mixture of 40 volumes of water R and 60 volumes of
180 acetonitrile R as diluent. For solution (1), dissolve 50.0 mg of the test substance in 30 mL of
181 acetonitrile and dilute to 50.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL.
182 Dilute 1.0 mL of this solution to 10.0 mL. For solution (3), dissolve 2 mg of estrone R (impurity
183 C) in 10.0 mL. Dilute 1.0 mL of this solution to 100.0 mL. For solution (4), dissolve the content
184 of a vial of ethinylestradiol for system suitability RS (containing ethinylestradiol and the
185 impurities B, F, H, I and K) in 1.0 mL of solution (3).

186
187 Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet
188 spectrophotometer set at a wavelength of 220 nm. Maintain the column temperature at 30 °C.
189 Inject alternatively 30 µL each of solution (1), (2) and (4) and record the chromatograms.

190
191 Use the chromatogram obtained with solution (4) and the chromatogram supplied with
192 ethinylestradiol for system suitability RS to identify the peaks due to the impurities B, C, F, H, I
193 and K. The impurities, if present, are eluted at the following relative retention with reference to
194 ethinylestradiol (retention time about 35 min): impurity F about 0.2; impurity H about 0.5;
195 impurity I about 0.8; impurity B about 0.88; impurity C about 0.92; impurity K about 1.3.

196
197 The test is not valid unless in the chromatogram obtained with solution (4) the resolution between
198 the peaks due to impurity I and B is at least 1.2.

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In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 0.7, is not greater than five times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.5 %);
- the area of any peak corresponding to impurity I, when multiplied by a correction factor of 0.4, is not greater than twice the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity H or K is not greater than twice the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity C or F is not greater than 1.5 times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.15 %);
- the area of any other impurity peak is not greater than the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.10 %);
- the sum of the corrected areas of any peak corresponding to impurity B and I and the areas of all other impurity peaks is not greater than eight times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.8 %). Disregard any peak with an area less than 0.5 times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.05 %).

Estrone. 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 92 volumes of dichloroethane R, 8 volumes of methanol R, and 0.5 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 freshly prepared solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 20 mg of the test substance per mL, and (B) 0.20 mg of estrone RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air until the

232 odour of the solvent is no longer detectable; then heat at 110°C for 10 minutes. Spray the hot plate
233 with sulfuric acid/ethanol TS, heat again at 110°C for 10 minutes, and examine the chromatogram
234 in ultraviolet light (365 nm). The spot obtained with solution B is more intense than any spot,
235 corresponding in position and appearance, obtained with solution A.

236

237 **Assay**

238

239 • Either method A or method B may be applied.

240

241 A. Carry out the test as described under 1.14.4 High-performance liquid chromatography,
242 using the conditions given below under “Related substances”.

243

244 Use solution (1) as described under “Related substances”. Prepare the following additional
245 solution: for solution (5), dissolve 50.0 mg of ethinylestradiol RS in 30 mL of acetonitrile R
246 and dilute to 50.0 mL with water R.

247

248 Inject alternately 50 µL each of solution (1) and (5) and record the chromatograms.

249

250 Measure the areas of the peaks corresponding to ethinylestradiol obtained in the
251 chromatograms of solutions (1) and (5) and calculate the percentage content of
252 ethinylestradiol (C₂₀H₂₄O₂) using the declared content of C₂₀H₂₄O₂ in ethinylestradiol RS.

253

254 B. Dissolve 50.0 mg of the test substance in sufficient dehydrated ethanol R and dilute to 100.0
255 mL with the same solvent. Dilute 10.0 mL of this solution to 50.0 mL with the same solvent.

256

257 Measure the absorbance of a 1 cm layer of the diluted solution at the maximum at about 281
258 nm. Calculate the percentage content of ethinylestradiol (C₂₀H₂₄O₂) using the absorptivity
259 value of 7.1 ($A_{1\text{ cm}}^{1\%} = 71$)

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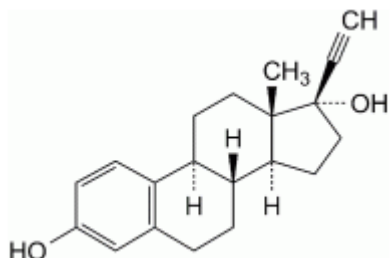
261 ~~Dissolve about 0.05 g, accurately weighed, in sufficient dehydrated ethanol R to produce~~
262 ~~100 mL, and dilute 10.0 mL of this solution to 50.0 mL with the same solvent. Measure the~~
263 ~~absorbance of a 1 cm layer of the diluted solution at the maximum at about 281 nm.~~

264 ~~Calculate the amount of C₂₀H₂₄O₂ in the substance being tested by comparison~~

265 with ethinylestradiol RS, similarly and concurrently examined. In an adequately calibrated
266 spectrophotometer the absorbance of the reference solution should be 0.72 ± 0.04 .

267

268 **Impurities**

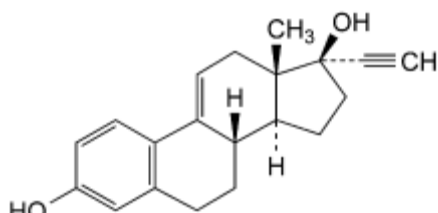


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271 A. 19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol (17β-ethinylestradiol)

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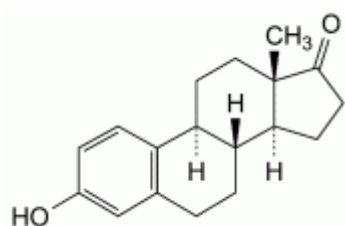


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275 B. 19-nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol (degradation product)

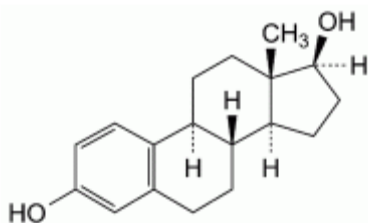
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279 C. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone) (synthesis related impurity, degradation
280 product)

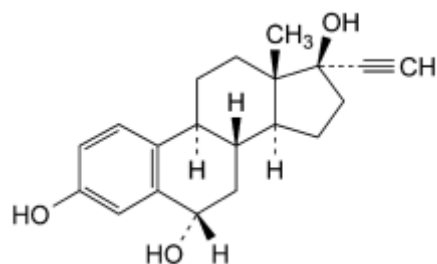


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283 D. *estra-1,3,5(10)-triene-3,17β-diol (estradiol) (degradation product)*

284

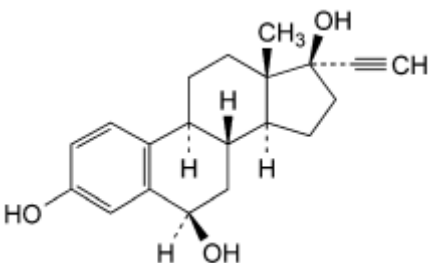


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287 E. *19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,6α,17-triol (6α-hydroxy-ethinylestradiol)*

288 (degradation product)

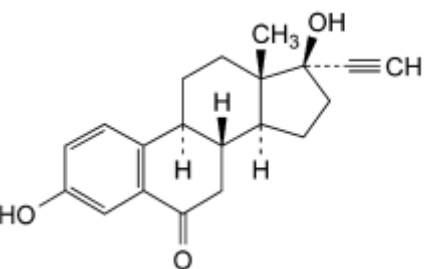


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291 F. *19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,6β,17-triol (6β-hydroxy-ethinylestradiol)*

292 (degradation product)

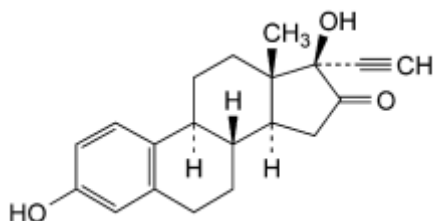


293

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295 G. 3,17-dihydroxy-19-nor-17 α -pregna-1,3,5(10)-trien-20-yn-6-one (6-oxo-ethinylestradiol)
296 (degradation product)

297

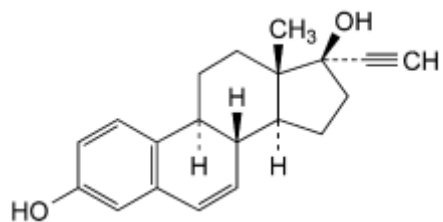


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300 H. 3,17-dihydroxy-19-nor-17 α -pregna-1,3,5(10)-trien-20-yn-16-one (16-oxo-ethinylestradiol)

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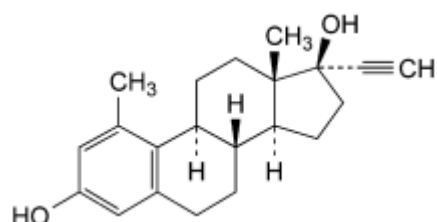


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304 I. 19-nor-17 α -pregna-1,3,5(10),6-tetraen-20-yne-3,17-diol

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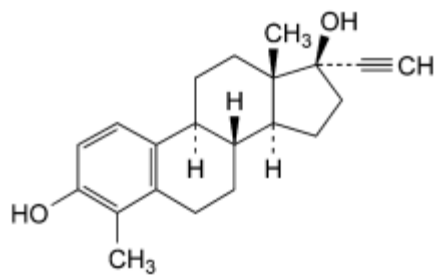


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308 J. 1-methyl-19-nor-17 α -pregna-1,3,5(10)-trien-20-yne-3,17-diol (1-methyl-ethinylestradiol)

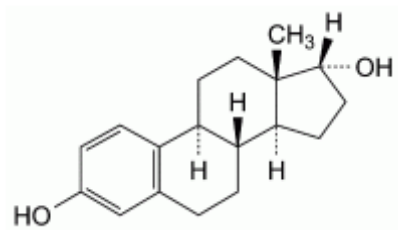
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311 K. 4-methyl-19-nor-17 α -pregna-1,3,5(10)-trien-20-yne-3,17-diol (4-methyl-ethinylestradiol)

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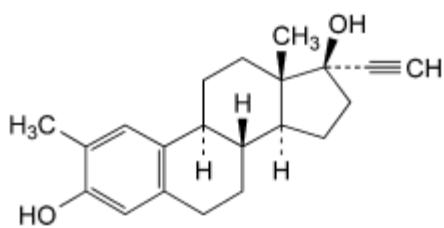


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315 L. *estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol)*

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319 M. *2-methyl-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol (2-methyl-ethinylestradiol)*

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