World Health Organization

REVISION OF THE MONOGRAPH ON ETHINYLESTRADIOL (ETHINYLESTRADIOLUM)

Draft proposal for inclusion in *The International Pharmacopoeia*

(December 2018)

DRAFT FOR COMMENTS

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.

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

Draft proposal for inclusion in *The International Pharmacopoeia*

REVISION OF THE MONOGRAPH ON ETHINYLESTRADIOL (ETHINYLESTRADIOLUM)

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.	

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

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

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

Changes from the current monograph are indicated in the text by <u>insert</u> or ~~delete~~.]

Draft proposal for inclusion in *The International Pharmacopoeia*

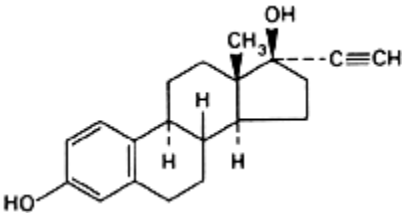
REVISION OF THE MONOGRAPH ON ETHINYLESTRADIOL (ETHINYLESTRADIOLUM)

Ethinylestradiol (Ethinylestradiolum)

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

Relative molecular mass. 296.4

Graphic formula.



Chemical name. 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol~~; 17-ethynyl-estra-1,3,5,(10)-triene-3,17β-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~~.

Requirements

Definition. Ethinylestradiol contains not less than 97.5~~97.0~~% and not more than 102.0% of $C_{20}H_{24}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A 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 ethinylestradiol RS or with the reference spectrum of ethinylestradiol.

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

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

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

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

Specific optical rotation. Use a 4.0 mg/mL solution in pyridine R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = -27.0° to -30.0°.

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

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

Use the following conditions for gradient elution:

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

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

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–35	100	0	Isocratic
35–65	100 to 0	0 to 100	Linear gradient
65–66	0 to 100	100 to 0	Return to initial composition
66–75	100	0	Re-equilibration

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

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

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

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

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~~

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

Assay

• Either method A or method B may be applied.

A. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using the conditions given below under "Related substances".

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

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

Measure the areas of the peaks corresponding to ethinylestradiol obtained in the chromatograms of solutions (1) and (5) and calculate the percentage content of ethinylestradiol ($C_{20}H_{24}O_2$) using the declared content of $C_{20}H_{24}O_2$ in ethinylestradiol RS.

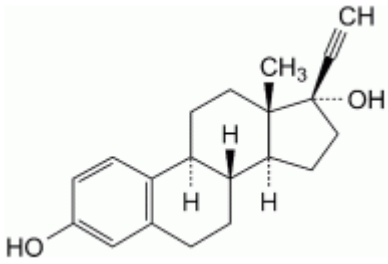
B. Dissolve 50.0 mg of the test substance in sufficient dehydrated ethanol R and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 50.0 mL with the same solvent.

Measure the absorbance of a 1 cm layer of the diluted solution at the maximum at about 281 nm. Calculate the percentage content of ethinylestradiol ($C_{20}H_{24}O_2$) using the absorptivity value of 7.1 ($A_{1\,cm}^{1\,\%}$ = 71)

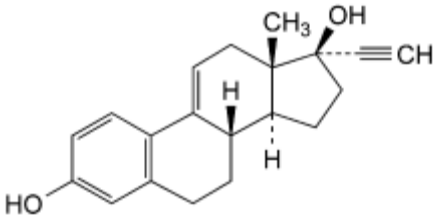
~~Dissolve about 0.05 g, accurately weighed, in sufficient dehydrated ethanol R to produce 100 mL, and dilute 10.0 mL of this solution to 50.0 mL with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 281 nm. Calculate the amount of $C_{20}H_{24}O_2$ in the substance being tested by comparison~~

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

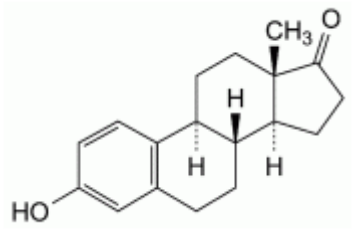
Impurities



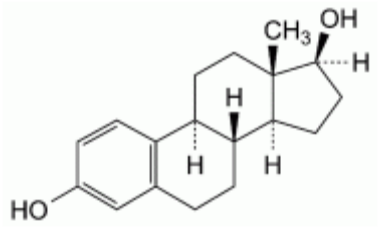
A. 19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol (17β-ethinylestradiol)



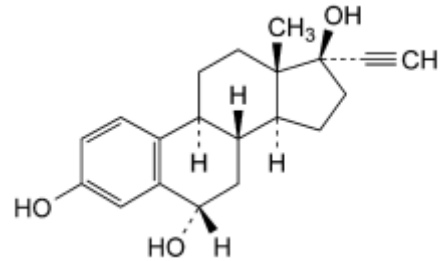
B. 19-nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol (degradation product)



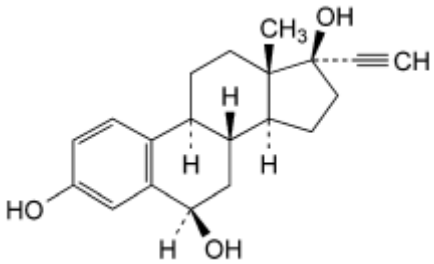
C. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone) (synthesis related impurity, degradation product)



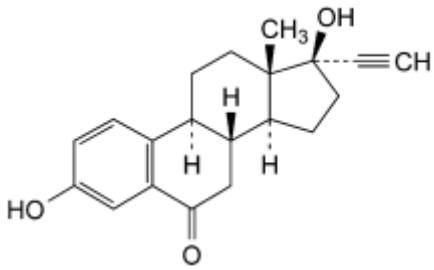
D. estra-1,3,5(10)-triene-3,17β-diol (estradiol) (degradation product)



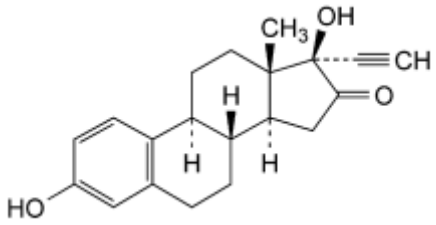
E. 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,6α,17-triol (6α-hydroxy-ethinylestradiol) (degradation product)



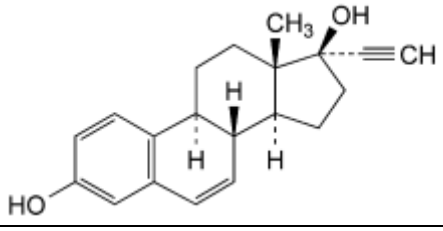
F. 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,6β,17-triol (6β-hydroxy-ethinylestradiol) (degradation product)



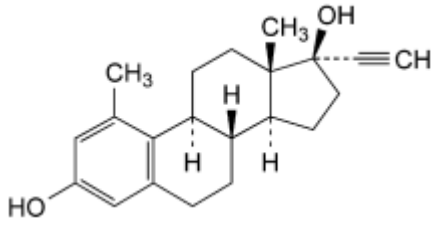
G. 3,17-dihydroxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-6-one (6-oxo-ethinylestradiol) (degradation product)



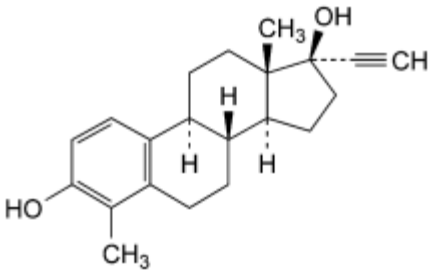
H. 3,17-dihydroxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-16-one (16-oxo-ethinylestradiol)



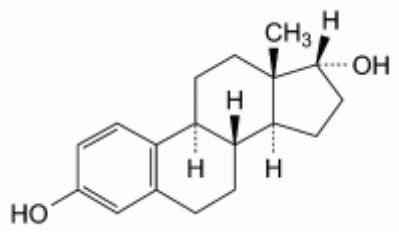
I. 19-nor-17α-pregna-1,3,5(10),6-tetraen-20-yne-3,17-diol



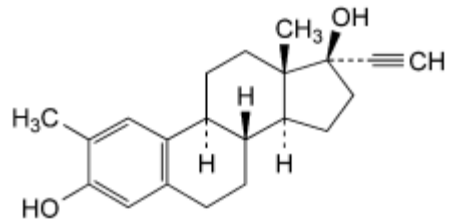
J. 1-methyl-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol (1-methyl-ethinylestradiol)



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



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



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

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