World Health Organization

ESTRADIOL VALERATE AND NORETHISTERONE ENANTATE INJECTION

(ESTRADIOLI VALERAS ET NORETHISTERONI ENANTAS INJECTIO)

Draft proposal for inclusion in *The International Pharmacopoeia*

(August 2023)

DRAFT FOR COMMENTS

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For any technical queries, please contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Sinéad Jones (jonessi@who.int, nsp@who.int).
Comments should be submitted through the online platform on or by **31 October 2023**. Please note that only comments received by this deadline will be considered for the preparation of this document.
Our working documents are sent out electronically and uploaded into PleaseReview™. The working documents are also placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/working-documents-public-consultation) under the "Working documents in public consultation". If you wish to receive all our draft guidelines during the course of the year, please send your full name, organization/ affiliation, and email address to jonessi@who.int, nsp@who.intand your name will be added to our electronic mailing list and review platform.

SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/23.941:

ESTRADIOL VALERATE AND NORETHISTERONE ENANTATE INJECTION

(ESTRADIOLI VALERAS ET NORETHISTERONI ENANTAS INJECTIO)

Submission of specifications and samples.	September 2018
First draft proposal.	September 2018
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2018
Discussion at the consultation on screening technologies, laboratory tools and pharmacopoeial specifications for medicines	2-3 May 2019
Presentation to WHO Expert Committee on Specification of Pharmaceutical Preparations	October 2019
Discussion at the consultation on screening technologies, laboratory tools and pharmacopoeial specifications for medicines	May 2020
Presentation to the WHO Expert Committee on Specification for Pharmaceutical Preparations	October 2020
Discussion at the consultation on screening technologies, laboratory tools and pharmacopoeial specifications for medicines	May 2021
Discussion at the Consultation on Quality Control and Pharmacopoeial specifications for medicines	April 2023
Draft monograph sent out for public consultation	September – October 2023
Presentation to the 57th meeting of the Expert Committee on Specifications for Pharmaceutical Preparations	October 2023
Further follow-up action as required.	

*[**Note from the Secretariat:** The draft monograph on Estradiol valerate and norethisterone enantate injection is proposed for inclusion in* The International Pharmacopoeia.

The proposed methods and specifications are based on a submission from a manufacturer and upon laboratory investigations.]

ESTRADIOL VALERATE AND NORETHISTERONE ENANTATE INJECTION (ESTRADIOLI VALERAS ET NORETHISTERONI ENANTAS INJECTIO)

Description. A clear, colourless or almost colourless, oily solution.

Category. Contraceptive.

Storage. Estradiol valerate and norethisterone enantate injection should be kept in a closed container, protected from light.

Labelling. The oil used in the formulation should be indicated.

Additional information. Strength in the eighth invitation to manufacturers of reproductive health products to submit an expression of interest (EOI) for product evaluation to the World Health Organization (WHO) Prequalification Team – Medicines: 5 mg/mL of Estradiol valerate and 50 mg/mL of Norethisterone enantate in 1 mL ampoule.

Requirements

Definition. Estradiol valerate and norethisterone enantate injection contains not less than 90.0% and not more than 110.0% of the amounts of Estradiol valerate ($C_{23}H_{32}O_3$) and Norethisterone enantate ($C_{27}H_{38}O_3$) as stated on the label.

Identity tests

- Either test A or test B may be applied:

A. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions as given under "Assay". The retention times of the peaks due to norethisterone enantate and estradiol valerate in the chromatogram obtained with solution (1) correspond to the retention times of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

B. Carry out the test as described under *1.14.1 Thin-layer chromatography* using silica gel R6 or equivalent as the coating substance and a mixture of 4 volumes of cyclohexane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µL of each of the following five solutions in methanol R. For solution (A), dilute 1.0 mL of the sample solution to 25.0 mL. For solution (B), dissolve 20 mg of norethisterone enantate RS and dilute to 10.0 mL. For solution (C), dissolve 2 mg of estradiol valerate RS and dilute to 10.0 mL. For solution (D), use a suitably diluted solution of the oil used in the formulation. For solution (E), dissolve 400 mg benzyl benzoate R in 25.0 mL. After removing the plate from the chromatographic chamber, allow it to air dry and examine the chromatogram in ultraviolet light (254 nm). Spray the plate with 4-toluenesulfonic acid/ethanol TS, heat at 120 °C for 10 minutes and examine the chromatogram in ultraviolet light (365 nm). The principal spots obtained with solution (A) correspond in position and appearance to the spots due to norethisterone enantate obtained with solution (B) and due to estradiol valerate obtained with solution (C). The chromatogram of solution (A) may show spots due to the oil used in the formulation or benzyl benzoate.

Bacterial endotoxins. Carry out the test as described under *3.4 Test for bacterial endotoxins*; contains not more than 1.5 IU of endotoxin RS per mg of norethisterone enantate.

Clarity and color of solution. Use 2.0 mL of the sample solution. This solution is clear and not more intensely coloured than reference solution Y3 or BY3 when compared as described under *1.11.2 Degree of coloration of liquids*, Method I.

Related substances. Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using a stainless steel column (5 cm × 2.1 mm) packed with particles of

silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (1.8 µm).[1]

Use the following conditions for gradient elution:

- mobile phase A: water R; and
- mobile phase B: acetonitrile for chromatography R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–2	67	33	Isocratic
2–17	67 to 35	33 to 65	Linear gradient
17–25	35 to 0	65 to 100	Linear gradient
25–30	0	100	Isocratic
30–30.1	0 to 67	100 to 33	Return to initial composition
30.1-33	67	33	Re-equilibration

Operate with a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 220 nm and 240 nm. Maintain the column temperature at 60°C.

Prepare the following solutions using as the diluent a mixture of 15 volumes of water R and 85 volumes of acetonitrile R. For solution (1), use solution (1) as described under "Assay". For solution (2) , dilute 5.0 mL of solution (1) to 500.0 mL. For solution (3) , dilute 10.0 mL of this solution to 100.0 mL. For solution (4), use a solution containing 0.20 mg of norethisterone caproate RS and 0.20 mg of norethisterone enantate RS per mL. For solution (5), use a suitably diluted solution of the oil used in the formulation. For solution (6), dissolve 0.25g benzyl benzoate R in 100.0 mL.

[1] An Agilent Zorbax SB-C18 column has been found suitable.

Inject 5 µL each of solutions (2), (3) and (4) and record the chromatograms.

The test is not valid unless, in the chromatogram obtained with solution (2), recorded at 220 nm, the signal-to-noise ratio of the peak due to estradiol valerate is at least 20. The test is also not valid unless, in the chromatogram obtained with solution (3), recorded at 240 nm, the signal-to-noise ratio of the peak due to norethisterone enantate is at least 10. In the chromatogram obtained with solution (4), recorded at 240 nm, the resolution between the peak due to norethisterone enantate impurity F (norethisterone capreoate) and the peak due to norethisterone enantate is at least 3.0.

Inject alternately 10 each of solutions (1), (2), (5) and (6) and record the chromatograms.

Use the chromatogram obtained with solutions (5) and (6) to identify any peaks due to the oil used in the formulation and benzyl benzoate, if present. Estradiol valerate is eluted with a retention time of about 13.5 minutes and norethisterone enantate with a retention time of about 17 minutes.

In the chromatogram obtained with test solution (1), recorded at 220 nm:

- the area of any impurity peak is not greater than 0.5 times the area of the peak due to estradiol valerate in the chromatogram obtained with solution (2), recorded at the same wavelength (0.5%).

In the chromatograms obtained with test solution (1), recorded at 240 nm:

- the area of any impurity peak is not greater than 0.5 times the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2), recorded at the same wavelength (0.5%).

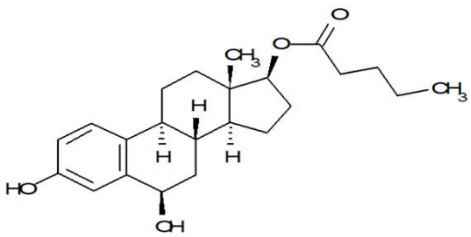
Assay. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the chromatographic conditions as described under “Related substances”.

Prepare the following solutions using as the diluent a mixture of 15 volumes of water R and 85 volumes of acetonitrile R. Weigh the contents of 10 vials, mix and determine the weight per Millilitre (*1.3.1*) of the solution. For solution (1), transfer a weighed quantity of the mixed contents, nominally equivalent to 75.0 mg of Norethisterone enantate, into a 250 mL volumetric flask. Add about 200 mL of the diluent, sonicate for 5 minutes, allow to cool to room temperature and dilute to volume. No oil droplets should be visible. For solution (2), dissolve 60.0 mg norethisterone enantate RS and dilute to 200.0 mL. For solution (3), dissolve 60.0 mg estradiol valerate RS and dilute to 200.0 mL. Dilute 10.0 mL of this solution to 100.0 mL.

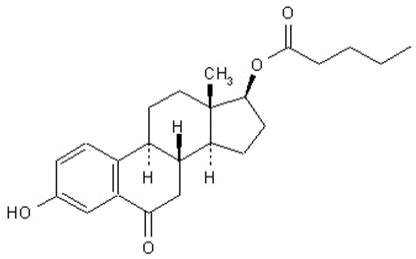
Inject alternately 5 µL each of solutions (1), (2) and (3) and record the chromatograms.

Measure the areas of the peaks corresponding to norestisterone enantate obtained in the chromatograms of solutions (1) and (2), recorded at 240 nm, and calculate the percentage content of $C_{27}H_{38}O_3$ per mL of the injection solution, using the declared content of $C_{27}H_{38}O_3$ in norethisterone enantate RS. Measure the areas of the peaks corresponding to estradiol valerate obtained in the chromatograms of solutions (1) and (3), recorded at 220 nm, and calculate the percentage content of $C_{23}H_{32}O_3$ per mL of the injection solution, using the declared content of $C_{23}H_{32}O_3$ in estradiol valerate RS.

Impurities. The impurities limited by the requirements of this monograph include those listed in the monographs on Norethisterone enantate and on Estradiol valerate and the following:



1. 3,6beta-Dihydroxyestra-1,3,5(10)-trien-17beta-yl-pentanoat (degradation product).



2. 3-Hydroxy-17beta-valeryloxyestra-1,3,5(10)-trien-6-on (degradation product).

Reference substances:

Estradiol valerate ICRS

New ICRS to be established.

Norethisterone enantate ICRS

New ICRS to be established.

Norethisterone caproate ICRS

New ICRS to be established.

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