Edition: BP 2025 (Ph. Eur. 11.6 update)

Estradiol and Norethisterone Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Estradiol and Norethisterone Tablets contain Estradiol Hemihydrate and Norethisterone. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$

95.0 to 105.0% of the stated amount.

Content of norethisterone, $C_{20}H_{26}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Add 0.2 mL of *water* to two tablets and shake to disperse. Add sufficient *ethanol (96%)* to produce a solution containing 0.035% w/v of Norethisterone, centrifuge and use the clear supernatant liquid.
(2) A suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 2 µL of each solution.
(d) Develop the plate to 8 cm.
(e) After removal of the plate, dry in air and spray with *ethanolic sulfuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of *dichloromethane*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two clearly separated spots with R_f values corresponding to those observed in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows two peaks having the same retention times as those due to estradiol and norethisterone in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
(b) Use 500 mL of a 0.3% w/v solution of *sodium lauryl sulfate* in *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter, discarding the first 5 mL of the filtrate. Use the filtered medium.
(2) Dissolve a sufficient quantity of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *methanol (80%)* and dilute an aliquot with a 0.3% w/v solution of *sodium lauryl sulfate* in *water*; the concentration of the final solution should be the same as that expected for solution (1).
(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 235 nm.
(f) Inject 200 µL of each solution.

MOBILE PHASE

450 volumes of *water* and 550 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, and norethisterone, $C_{20}H_{26}O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

Estrone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Powder 20 tablets. Add 20 mL of the mobile phase to a quantity of the powdered tablets containing the equivalent of 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 mL. Centrifuge and use the clear supernatant liquid.
(2) 0.0001% w/v of *estrone BPCRS* in the mobile phase.
(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to estrone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Uniformity of content

Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of estradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of the mobile phase to one tablet, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 mL and centrifuge. Dilute the supernatant liquid, if necessary, with the mobile phase to produce a solution containing the equivalent of 0.002% w/v of estradiol.
(2) Dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for solution (1).
(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ and $C_{20}H_{26}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

ASSAY

For estradiol

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of estradiol

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of estradiol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of the mobile phase to a quantity of powdered tablets containing the equivalent of 10 mg of estradiol, mix with the aid of ultrasound and centrifuge. Dilute 1 volume of the clear supernatant liquid to 10 volumes with mobile phase.
(2) 0.002% w/v of *estradiol hemihydrate BPCRS* in the mobile phase.
(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, in the tablets using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Estradiol and Norethisterone Tablets should be protected from light.

LABELLING

The quantity of Estradiol Hemihydrate is stated in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone.