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Estradiol and Norethisterone Acetate Tablets

General Notices

Action and use

Estrogen.

DEFINITION

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

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

Content of estradiol, $C_{18}H_{24}O_2$ and norethisterone acetate, $C_{22}H_{28}O_3$

Content		Limits	
Estradiol mg	Norethisterone Acetate mg	Estradiol % of stated amount	Norethisterone Acetate % of stated amount
2	1	95.0 to 105.0	92.5 to 105.0
1	1	95.0 to 105.0	92.5 to 105.0
1	0.5	95.0 to 105.0	92.5 to 105.0
0.5	0.1	95.0 to 105.0	90.0 to 105.0

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 Acetate, centrifuge and use the clear supernatant liquid.
(2) A suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone acetate 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 15 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*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated spots.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those 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 the peaks due to estradiol and norethisterone acetate 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*, 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. Use the filtered medium, diluted with a 0.3% w/v solution of *sodium lauryl sulfate* if necessary, to produce a solution expected to contain 0.0001% w/v of estradiol.
(2) Dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in a 0.3% w/v solution of *sodium lauryl sulfate* and dilute with a sufficient volume of a 0.3% w/v solution of *sodium lauryl sulfate* to produce concentrations in the final solution the same as those expected for solution (1).

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 (2), 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 of norethisterone acetate, $C_{22}H_{28}O_3$, in the medium using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

Estrone and norethisterone

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

(1) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 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) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 2.5 mg of Norethisterone Acetate, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 mL. Centrifuge and use the clear supernatant liquid.
(3) 0.0001% w/v of *estrone BPCRS*.
(4) 0.00005% w/v of *norethisterone BPCRS*.
(5) 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*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but injecting 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), 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) quantify the area of any peak due to estrone using the principal peak in the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (2) quantify the area of any peak due to norethisterone using the principal peak in the chromatogram obtained with solution (4).

Content of		Limits	
Estradiol mg	Norethisterone Acetate mg	Estrone %	Norethisterone %
2	1	0.5	0.5
1	1	0.5	0.5
1	0.5	0.5	0.5
0.5	0.1	0.5	1.0

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of estradiol or less than 2 mg and/or less than 2% w/w of Norethisterone Acetate 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 acetate BPCRS* in the mobile phase and dilute with sufficient mobile phase to produce concentrations in the final solution 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 injecting 20 µL of each solution.

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 of $C_{22}H_{28}O_3$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

ASSAY

For estradiol

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

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 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 in the mobile phase.

(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 10 mL of the clear supernatant liquid to 100 mL with the mobile phase.
(2) 0.002% w/v of *estradiol hemihydrate BPCRS*.
(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*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but injecting 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), 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 $C_{18}H_{24}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone acetate

For tablets containing 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.

STORAGE

Estradiol and Norethisterone Acetate Tablets should be protected from light.

LABELLING

The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone,
B. Norethisterone.