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Estradiol and Norethindrone Acetate Tablets

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-estradiol-norethindrone-acetate-tabs-20230331.

DEFINITION

Estradiol and Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

IDENTIFICATION

- **A.** The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile and water (55:45)

Diluent: Dehydrated alcohol and water (50:50)

Estrone standard stock solution: 0.12 mg/mL of USP Estrone RS in dehydrated alcohol

Estradiol standard stock solution: 0.25 mg/mL of USP Estradiol RS in dehydrated alcohol

Norethindrone acetate standard stock solution: 0.15 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol

System suitability solution: Combine 800 µL of the *Estradiol standard stock solution*, 600 µL of the *Norethindrone acetate standard stock solution*, 200 µL of the *Estrone standard stock solution*, and 10.0 mL of *Diluent*.

Standard solution: 20 µg/mL of USP Estradiol RS from the *Estradiol standard stock solution* and 10 µg/mL of USP Norethindrone Acetate RS from the *Norethindrone acetate standard stock solution* in *Diluent*

Sample solution: Nominally 20 µg/mL of estradiol and 10 µg/mL of norethindrone acetate from Tablets (NLT 12) in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV dual wavelength (254 nm/280 nm) or equivalent. For *Identification A*, use a diode array detector in the range of 190–400 nm. [Note—The absorption of estradiol at 280 nm and norethindrone acetate at 254 nm can be included in a single run by altering the wavelength.]

Column: 4.6-mm × 15-cm; 4-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 4 times the retention time of estradiol

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.8 between estradiol and estrone, *System suitability solution*

Relative standard deviation: NMT 3%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estradiol ($C_{18}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response at 280 nm of estradiol from the *Sample solution*

r_S = peak response at 280 nm of estradiol from the *Standard solution*

C_S = concentration of USP Estradiol RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of estradiol in the *Sample solution* (µg/mL)

Calculate the percentage of labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response at 254 nm of norethindrone acetate from the *Sample solution*

r_S = peak response at 254 nm of norethindrone acetate from the *Standard solution*

C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of norethindrone acetate in the *Sample solution* (µg/mL)

Acceptance criteria

Estradiol: 90.0%–110.0%

Norethindrone acetate: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Test 1

Medium: 0.3% sodium lauryl sulfate; 500 mL

Apparatus 2: 50 rpm

Times: 30 min for Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate, and 50 min for Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate

Mobile phase: Acetonitrile and water (55:45)

Standard stock solution A: 20 µg/mL of USP Estradiol RS in alcohol or in a mixture of alcohol and water

Standard stock solution B: 10 µg/mL of USP Norethindrone Acetate RS in alcohol or in a mixture of alcohol and water

Standard solution: Dilute suitable quantities of *Standard stock solution A* and *Standard stock solution B* in *Medium* or a mixture of alcohol and water to obtain a final concentration of both analytes similar to the expected concentration of the *Sample solution*.

Sample solution: Pass a portion of the solution through a filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 241 nm for norethindrone acetate and 280 nm for estradiol

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 150 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of estradiol or norethindrone acetate from the *Sample solution*

r_S = peak response of estradiol or norethindrone acetate from the *Standard solution*

C_S = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim of estradiol or norethindrone acetate (mg/Tablet)

Tolerances: For Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate: NLT 75% (*Q*) of the labeled amounts of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) are dissolved in 30 min. For Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate: NLT 75% (*Q*) of the labeled amounts of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) are dissolved in 50 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.3% sodium lauryl sulfate; 500 mL, ▲deaerated, if necessary▲ (RB 1-Apr-2023)

Apparatus 2: 50 rpm

Time: 20 min

Mobile phase: Acetonitrile and water (55:45)

Standard stock solution A: 0.5 mg/mL of USP Estradiol RS in methanol. Sonicate as necessary.

Standard stock solution B: 0.5 mg/mL of USP Norethindrone Acetate RS in methanol. Sonicate as necessary.

Standard solution: (L_1/500) mg/mL of USP Estradiol RS and (L_2/500) mg/mL of USP Norethindrone Acetate RS prepared from *Standard stock solution A* and *Standard stock solution B* in *Medium*, where L_1 is the label claim of estradiol and L_2 is the label claim of norethindrone acetate, in mg/Tablet

Sample solution: Pass a portion of the solution through a filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 241 nm for norethindrone acetate and 210 nm for estradiol

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 20°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 1.3 times the retention time of norethindrone acetate

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for estradiol and norethindrone acetate

Relative standard deviation: NMT 2.0% for estradiol and norethindrone acetate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of estradiol or norethindrone acetate from the *Sample solution*

r_S = peak response of estradiol or norethindrone acetate from the *Standard solution*

C_S = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim of estradiol or norethindrone acetate (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amounts of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) are dissolved in 20 min.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A: Tetrahydrofuran and water (1:200)

Solution B: Acetonitrile, tetrahydrofuran, and water (160:1:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	65	35
35	20	80
49	20	80
50	80	20

Time (min)	Solution A (%)	Solution B (%)
60	80	20

Diluent: Dehydrated alcohol and water (50:50)

System suitability solution: 240 µg/mL of USP Estradiol RS, 60 µg/mL of USP Norethindrone Acetate RS, and 1 µg/mL of USP Estrone RS in *Diluent*

Estradiol standard stock solution: 250 µg/mL of USP Estradiol RS in alcohol

Norethindrone acetate standard stock solution: 150 µg/mL of USP Norethindrone Acetate RS in alcohol

Standard solution: Combine 250 µL of the *Estradiol standard stock solution* and 100 µL of the *Norethindrone acetate standard stock solution*, and dilute with 50.0 mL of *Diluent*.

Sample solution: Nominally 240 µg/mL of estradiol and 120 µg/mL of norethindrone acetate from Tablets (NLT 12) in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 235 and 254 nm

Column: 3.9-mm × 30-cm; 4-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for estradiol, estrone, and norethindrone acetate are about 1.0, 1.1, and 1.7, respectively.]

Suitability requirements

Resolution: NLT 1.3 between estrone and estradiol, measured at 254 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of any estradiol related impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 235 nm for each impurity from the *Sample solution*

r_S = peak response at 235 nm from the *Standard solution*

C_S = concentration of the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2* or *Table 3*)

Calculate the percentages of any norethindrone acetate related impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 254 nm for each impurity from the *Sample solution*

r_S = peak response at 254 nm from the *Standard solution*

C_S = concentration of the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2* or *Table 3*)

Acceptance criteria: The Tablets meet the requirements given in *Table 2* or *Table 3*. See *Table 2* for Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate or *Table 3* for Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate.

Table 2. For Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6α-Hydroxyestradiol[a]	0.47	1.0	1.0
6β-Hydroxyestradiol[b]	0.51	1.0	1.0
Estradiol related compound C[c]	0.62	1.0	1.0
Estradiol related compound B[d]	0.95	1.0	1.0
Estradiol	1.0	—	—
Any estradiol related unspecified impurity	—	1.0	0.5
Total estradiol related impurities	—	—	2.0
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate[e]	0.58	1.0	1.0
Norethindrone[f]	0.66	1.0	1.0
6-Ketonorethindrone acetate[g]	0.79	0.56	1.0
6-Dehydronorethindrone acetate[h]	0.97	0.45	1.0
Norethindrone acetate	1.0	—	—
Any norethindrone acetate related unspecified impurity	—	1.0	0.5
Total norethindrone acetate related impurities	—	—	2.0

[a] 1,3,5(10)-Estratriene-3,6α,17β-triol.

[b] 1,3,5(10)-Estratriene-3,6β,17β-triol.

[c] 1,3,5(10)-Estratrien-3,17β-diol-6 one.

[d] 1,3,5(10),6-Estratetraen-3,17β-diol.

[e] 6β-Hydroxy-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.

[f] 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one.

[g] 3,6-Dioxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate

[h] 17-Hydroxy-19-nor-17α-pregn-4,6-dien-20-yn-3-one acetate.

Table 3. For Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6β-Hydroxyestradiol[a]	0.51	1.0	1.0
Estradiol	1.0	—	—

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any estradiol related unspecified impurity	—	1.0	1.0
Total estradiol related impurities	—	—	2.5
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate[b]	0.58	1.0	1.5
Norethindrone[c]	0.66	1.0	1.0
6-Ketonorethindrone acetate[d]	0.79	0.56	2.5
6-Dehydronorethindrone acetate[e]	0.97	0.45	1.0
Norethindrone acetate	1.0	—	—
Any norethindrone acetate related unspecified impurity	—	1.0	1.0
Total norethindrone acetate related impurities	—	—	4.0

[a] 1,3,5(10)-Estratrien-3,6β,17β-triol.

[b] 6β-Hydroxy-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate

[c] 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one.

[d] 3,6-Dioxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.

[e] 17-Hydroxy-19-nor-17α-pregn-4,6-dien-20-yn-3-one acetate.

SPECIFIC TESTS

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is NMT 10^3 cfu/g. The total combined molds and yeasts count is NMT 10^2 cfu/g. Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP Reference Standards ⟨11⟩**

USP Estradiol RS

USP Estrone RS

USP Norethindrone Acetate RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ESTRADIOL AND NORETHINDRONE ACETATE TABLETS	Documentary Standards Support	SM52020 Small Molecules 5

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