

Status: Currently Official on 14-Feb-2025
 Official Date: Official as of 01-Apr-2023
 Document Type: USP Monographs
 DocId: GUID-BDBC3F2-B491-49C7-9AE1-AC03BB0FB873_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M30615_03_01
 DOI Ref: 6r35y

© 2025 USPC
 Do not distribute

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- μ m 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 $\times$ 15-cm; 5- μ m packing [L1](#)

Temperatures

Autosampler: 20°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 100 μ L

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



Documents

Document Sharing Hub