Norethindrone and Ethinyl Estradiol Tablets

Type of Posting	Revision Bulletin
Posting Date	27–Oct–2017
Official Date	01–Nov–2017
Expert Committee	Chemical Medicines Monographs 5
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Chemical Medicines Monographs 5 Expert Committee has revised the Norethindrone and Ethinyl Estradiol Tablets monograph. The purpose for the revision is to add dissolution tests to accommodate drug products which were approved with different conditions and acceptance criteria.

- Dissolution Test 2 was validated using a Hypersil BDS C18 brand of 4.6-mm x 10-cm, 3-µm packing L1 column. The typical retention times for Norethindrone and Ethinyl Estradiol are 5.4 and 6 min; respectively.
- Dissolution Test 3 was validated using a Hypersil BDS C18 brand of 4.6-mm x 10-cm, 3-µm packing L1 column. The typical retention times for Norethindrone and Ethinyl Estradiol are 6 and 7 min; respectively.

The Norethindrone and Ethinyl Estradiol Tablets Revision Bulletin supersedes the currently official Norethindrone and Ethinyl Estradiol Tablets monograph. The Revision Bulletin will be incorporated into the *Second Supplement* to *USP 41–NF 36*.

Should you have any questions, please contact Ren-Hwa Yeh, Ph.D., Senior Scientific Liaison, (301–998–6818 or rhy@usp.org).

Norethindrone and Ethinyl Estradiol Tablets

DEFINITION

Norethindrone and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Test**

Standard solution: 1 mg/mL of USP Norethindrone RS and 50 µg/mL of USP Ethinyl Estradiol RS in alcohol

Sample solution: Crush 1 Tablet in 1 mL of alcohol in a 15-mL conical centrifuge tube, and warm to 50° for 10 min with gentle swirling. Cool, and centrifuge to obtain a clear solution.

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 min

Application volume: 20 µL

Developing solvent system: Toluene and ethyl acetate (4:1)

Spray reagent: Sulfuric acid–methanol, prepared by cautiously adding 70 mL of sulfuric acid in small increments to 30 mL of methanol chilled in an ice-bath, and mixing

Analysis

Samples: *Standard solution* and *Sample solution*

Apply both *Standard solution* and *Sample solution* at a line 2.5 cm from the bottom of a thin-layer chromatographic plate. Develop the chromatogram in *Developing solvent system* until the solvent front has moved 10 cm. Remove the plate, and air-dry. Spray the plate with *Spray reagent*.

Acceptance criteria: The spots from the *Sample solution* have the same R_F values as the spots from USP Ethinyl Estradiol RS and from USP Norethindrone RS.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and water (60:40)

Diluent: Acetonitrile and water (45:55)

Internal standard solution: Transfer 15 mg of valerophenone into a 250-mL volumetric flask, add 125 mL of acetonitrile, and dilute with water to volume.

Ethinyl estradiol standard stock solution: 0.09 mg/mL of USP Ethinyl Estradiol RS in acetonitrile

Norethindrone standard stock solution: 1.25 mg/mL of USP Norethindrone RS in acetonitrile

Standard solution: Transfer 5.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add volumes of *Ethinyl estradiol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg/mL, of the Reference Standards correspond numerically to one-twentieth of the labeled amounts of the corresponding ingredients in the Tablets. Add (26 − *X*) mL of acetonitrile, *X* being the total volume of the standard stock solution taken. Dilute with *Diluent* to volume.

Sample stock solution: Transfer 10 Tablets to a 100-mL volumetric flask, add 20 mL of water, and shake by mechanical means until the Tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 60 mL of acetonitrile, and mix. Sonicate for 2 min. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary to obtain a slightly turbid solution.

Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Diluent* to 10.0 mL.

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and norethindrone are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between norethindrone and ethinyl estradiol

Column efficiency: NLT 8000 theoretical plates from the internal standard peak

Relative standard deviation: NMT 2.0% for six replicate injections for norethindrone and ethinyl estradiol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) in each Tablet:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio, at corresponding retention times, of the *Sample solution*

R_S = peak response ratio, at corresponding retention times, of the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding sample in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and 90.0%–110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$)

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

[NOTE—Exercise care in filtering and handling solutions containing ethinyl estradiol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytef pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytef-coated or solid polytef paddles.]

•**Test 1**• (RB 1-Nov-2017)

Medium: 0.09% sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Buffer: 0.02 M pH 6.0 phosphate buffer

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: USP Norethindrone RS and USP Ethinyl Estradiol RS in *Medium* with concentrations similar to those expected in the solution under test.

[NOTE—A volume of methanol not exceeding 4% of the total final volume of the *Standard solution* may be used in preparing the *Standard solution*.]

Sample solution: A filtered portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 5-mm × 8.3-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between norethindrone and ethinyl estradiol
Column efficiency: NLT 7000 theoretical plates for the ethinyl estradiol peak
Tailing factor: NMT 2.0 for either peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by comparison of the corresponding peak responses of the *Standard solution* and the solution under test.
Tolerances: NLT 75% (*Q*) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 60 min.

•**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.09% (w/v) sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 0.02 M pH 6.0 phosphate buffer prepared as follows. Dissolve 2.72 g of potassium phosphate, monobasic in 1 L of water. Adjust with 10% potassium hydroxide solution in water to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (42:58)
Standard stock solution 1: 100 µg/mL of USP Norethindrone RS in acetonitrile. Sonicate to dissolve as needed.
Standard stock solution 2: 3.5 µg/mL of USP Ethinyl Estradiol RS in acetonitrile. Sonicate to dissolve as needed.
Standard solution: 0.8 µg/mL of USP Norethindrone RS and 0.07 µg/mL of USP Ethinyl Estradiol RS in *Medium*, from *Standard stock solution 1* and *Standard stock solution 2*.
Sample solutions: Centrifuge a portion of the solution under test at about 3000 rpm for 3 min, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of ethinyl estradiol
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between norethindrone and ethinyl estradiol
Tailing factor: NMT 2.0 for norethindrone and ethinyl estradiol
Relative standard deviation: NMT 3.0% for norethindrone and ethinyl estradiol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

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

r_U = peak response of norethindrone or ethinyl estradiol from the *Sample solution*
r_S = peak response of corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
V = volume of *Medium*, 500 mL
L = label claim of norethindrone or ethinyl estradiol (µg/Tablet)
Tolerances: NLT 80% (*Q*) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 30 min.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.09% (w/v) sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 0.02 M pH 6.0 phosphate buffer prepared as follows. Dissolve 2.72 g of potassium phosphate, monobasic in 1 L of water. Adjust with 0.1 N sodium hydroxide solution in water to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (38:62)
Norethindrone standard stock solution: 0.8 mg/mL of USP Norethindrone RS in methanol. Sonicate to dissolve as needed.
Ethinyl estradiol standard stock solution: 0.135 mg/mL of USP Ethinyl Estradiol RS in methanol. Sonicate to dissolve as needed.
Standard solution: USP Norethindrone RS and USP Ethinyl Estradiol RS in *Medium* with concentrations similar to those expected in the solution under test, from *Norethindrone standard stock solution* and *Ethinyl estradiol standard stock solution*. Add intermediate diluting steps as needed.
Sample solution: Pass a portion of the solution under test through a filter of 0.45-µm pore size and use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 1.4 times the retention time of ethinyl estradiol
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for norethindrone and ethinyl estradiol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between norethindrone and ethinyl estradiol
Tailing factor: NMT 2.0 for norethindrone and ethinyl estradiol

Relative standard deviation: NMT 3.0% for norethindrone and ethinyl estradiol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

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

r_U = peak response of norethindrone or ethinyl estradiol from the *Sample solution*
r_S = peak response of corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
V = volume of *Medium*, 500 mL
L = label claim of norethindrone or ethinyl estradiol (µg/Tablet)

Tolerances: NLT 80% (Q) of each of the labeled amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$), respectively, are dissolved in 30 min. ● (RB 1-Nov-2017)

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩,** *Content Uniformity*: Meet the requirements with respect to norethindrone and to ethinyl estradiol

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Add the following:

●• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ● (RB 1-Nov-2017)

- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Ethinyl Estradiol RS
  USP Norethindrone RS