In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Chemical Medicines Monographs 5 Expert Committee has revised the Estradiol and Norethindrone Acetate Tablets monograph. The purpose for the revision is to add **Dissolution Test 2** to accommodate the FDA approved specifications for the sponsor product. The labeling information is also incorporated to support the inclusion of **Dissolution Test 2**.

- **Dissolution Test 2** was validated using Zorbax Eclipse XDB-C18 brand of L1 column. The typical retention times for estradiol and norethindrone acetate are about 3.0 min and 9.5 min respectively.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

The Estradiol and Norethindrone Acetate Tablets Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in the *Second Supplement to USP 40–NF 35*.

Should you have any questions, please contact Sridevi Ramachandran, PhD., Associate Scientific Liaison (*sdr@usp.org*) or Gerald Hsu, PhD., Senior Scientific Liaison (*gdh@usp.org*).
Estradiol and Norethindrone Acetate Tablets

**DEFINITION**
Estradiol and Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol (C18H24O2) and NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate (C22H28O3).

**IDENTIFICATION**

• **A. Thin-Layer Chromatographic Identification Test**
  (201)
  **Standard solution:** 0.5 mg/mL of USP Estradiol RS and 0.25 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol
  **Sample solution:** Place 2 Tablets into a 10-mL vial, and add 0.2 mL of water. When the Tablets are partially disintegrated, add a few glass beads, and shake vigorously to disintegrate. Add 4.0 mL of dehydrated alcohol, and shake. [NOTE—Centrifuge until the supernatant is clear before application to the plate.]
  **Adsorbent:** 0.25-mm chromatographic silica gel plate
  **Application volume:** 2 µL
  **Developing solvent system:** Chloroform and acetone each of the Tablets taken (9:1)
  **Column:** single run by altering the wavelength.
  **Injection size:** 50 µL

**ASSAY**

• **Procedure**
  **Mobile phase:** Acetonitrile and water (11:9)
  **Diluent:** Dehydrated alcohol and water (1:1)
  **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
  **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
  **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.
  **Sample solution:** Add 12 Tablets into a measured amount of Diluent to obtain a solution having an estradiol concentration of 20 µg/mL and a norethindrone acetate concentration of 10 µg/mL.
  **Chromatographic system** (See Chromatography (621), System Suitability.)

**Mode:** LC
**Detector:** UV dual wavelength (254 nm/280 nm) or equivalent
[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; packing L1
**Flow rate:** 1 mL/min
[NOTE—Perform an investigational run to determine the retention times for estradiol and norethindrone acetate.]
**Injection size:** 50 µL

**System suitability**

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

**Suitability requirements**

**Resolution:** NLT 1.8 between estradiol and estrone,
**Relative standard deviation:** NMT 3%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution
[NOTE—Measure the areas for the estradiol and norethindrone acetate peaks.]
Calculate the quantity, as a percentage, of C18H24O2 in each of the Tablets taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \times \frac{C_U}{C_S} \right) \times 100
\]

\[
r_U = \text{peak area from the Sample solution}
\]
\[
r_S = \text{peak area from the Standard solution}
\]
\[
C_U = \text{concentration of USP Estradiol RS in the Standard solution (µg/mL)}
\]
\[
C_S = \text{nominal concentration of estradiol in the Sample solution (µg/mL)}
\]

Calculate the quantity, as a percentage, of C22H28O3 in each of the Tablets taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \times \frac{C_U}{C_S} \right) \times 100
\]

\[
r_U = \text{peak area from the Sample solution}
\]
\[
r_S = \text{peak area from the Standard solution}
\]
\[
C_U = \text{concentration of USP Norethindrone Acetate RS in the Standard solution (µg/mL)}
\]
\[
C_S = \text{nominal concentration of norethindrone acetate in the Sample solution (µg/mL)}
\]

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of C18H24O2 and 90.0%–110.0% of the labeled amount of C22H28O3

**PERFORMANCE TESTS**

**Change to read:**

• **Dissolution (711)**
  **Test 1:** (88-1 Dec-2016)
  **Medium:** 0.3% sodium lauryl sulfate; 500 mL
  **Apparatus 1:** 50 rpm
  **Time:** 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 (11:9)
  **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 percentage of C₁₈H₂₄O₂ and of C₂₂H₂₈O₃ dissolved:

\[
\text{Result} = \frac{r_U}{r_S} \times \left( \frac{C_S}{C_U} \right) \times 100
\]

\( r_U = \) 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)

\( C_U = \) nominal concentration of estradiol or norethindrone acetate in the Sample solution (mg/mL) (based on the label claim)

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 C₁₈H₂₄O₂ and C₂₂H₂₈O₃ is 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 C₁₈H₂₄O₂ and C₂₂H₂₈O₃ is 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

Apparatus 2: 30 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: (L₁/500) mg/mL of USP Estradiol RS and (L₂/500) mg/mL of USP Norethindrone Acetate RS prepared from Standard stock solution A and Standard stock solution B in Medium, where L₁ is the label claim of estradiol and L₂ 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: NMT 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 percentage of the labeled amount of estradiol (C₁₈H₂₄O₂) and norethindrone acetate (C₂₂H₂₈O₃) dissolved:

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

\( r_U = \) 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 amount of estradiol (C₁₈H₂₄O₂) and norethindrone acetate (C₂₂H₂₈O₃) is dissolved in 20 min.

- Uniformity of Dosage Units (905): Meet the requirements

IMPURITIES

Organic Impurities

- Procedure

  Solution A: Tetrahydrofuran and water (1:200)

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

  Mobile phase: See the gradient table below.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>49</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>50</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>60</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Diluent: Dehydrated alcohol and water (1:1)

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: A quantity equivalent to 240 µg/mL of estradiol and 120 µg/mL of norethindrone acetate from NLT 20 finely ground Tablets 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 size: 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 requirementsResolution: NLT 1.3 between estrone and estradiol, measured at 254 nm

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of any estradiol impurity in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times (1/F) \times 100
\]

\[r_U = \text{peak response area at 235 nm for each impurity from the Sample solution}\]
\[r_S = \text{peak response area at 235 nm from the Standard solution}\]
\[C_S = \text{concentration of the Standard solution (µg/mL)}\]
\[C_U = \text{concentration of the Sample solution (µg/mL)}\]
\[F = \text{relative response factor (see Impurity Table 1 or Impurity Table 2)}\]

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

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times (1/F) \times 100
\]

\[r_U = \text{peak response area at 254 nm for each impurity from the Sample solution}\]
\[r_S = \text{peak response area at 254 nm from the Standard solution}\]
\[C_S = \text{concentration of the Standard solution (µg/mL)}\]
\[C_U = \text{concentration of the Sample solution (µg/mL)}\]
\[F = \text{relative response factor (Impurity Table 1 or Impurity Table 2)}\]

Acceptance criteria: The Tablets meet the requirements given in either Impurity Table 1 or Impurity Table 2.

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol related impurities</td>
<td>0.47</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6β-Hydroxyestradiol</td>
<td>0.51</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6-Ketosteradiol</td>
<td>0.62</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6-Dehydrosteradiol</td>
<td>0.95</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Any other single estradiol related impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Total estradiol related impurities</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol related impurities</td>
<td>0.58</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Norethindrone</td>
<td>0.66</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6-Ketonestradiol</td>
<td>0.79</td>
<td>0.56</td>
<td>1.0</td>
</tr>
<tr>
<td>6-Dehydronestradiol</td>
<td>0.97</td>
<td>0.45</td>
<td>1.0</td>
</tr>
<tr>
<td>Norethindrone acetate</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Any other single norethindrone acetate related impurity</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total norethindrone acetate related impurities</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Impurity Table 3. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate (Continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norethindrone acetate</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Specific Tests

- **Microbial Enumeration Tests (61) and Tests for Specified Microorganisms (62):** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g.
g. The Tablets meet 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.

**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-Dec-2016)

**USP Reference Standards (11)**
- USP Estradiol RS
- USP Estrone RS
- USP Norethindrone Acetate RS