Sensitivity solution: 1 µg/mL of doxycycline from the *Standard solution*
System suitability stock solution: 0.04 mg/mL each of USP Oxytetracycline Hydrochloride RS, USP Methacycline Hydrochloride RS, and USP Doxycycline Related Compound A RS
System suitability solution: Transfer 5 mL of the *Standard stock solution* into a 25-mL volumetric flask. Heat on a steam bath for 60 min, and gently evaporate to dryness on a hot plate (partial degradation of doxycycline to 4-epidoxycycline). Add 3 mL of the *System suitability stock solution* to the flask, and dilute with water to volume. Pass through a suitable filter.
System suitability
Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 10 for doxycycline, *Sensitivity solution*
Resolution: NLT 1.5 between doxycycline and 6-epidoxycycline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
The run time is 1.7 times the retention time of the doxycycline peak.
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity 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 of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)
Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxytetracycline	0.3	1.0	0.5
4-Epidoxycycline[a]	0.4	1.0	1.0
Methacycline	0.6	1.0	2.0
6-Epidoxycycline (doxycycline related compound A)[b]	0.7	0.86	2.0
Doxycycline	1.0	—	—

[a] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate.
[b] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

Add the following:

•• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.• (RB 1-Sep-2011)

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Doxycycline Hyclate RS
USP Doxycycline Related Compound A RS
6-Epidoxycycline, or (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.
$C_{22}H_{24}N_2O_8$ 444.43
USP Methacycline Hydrochloride RS
USP Oxytetracycline Hydrochloride RS

Add the following:

▪Drospirenone and Ethinyl Estradiol Tablets

DEFINITION

Drospirenone and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of drospirenone ($C_{24}H_{30}O_3$) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

• **A.** The retention times of the drospirenone and ethinyl estradiol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: Dissolve 132 g of dibasic ammonium phosphate in 0.8 L of water, adjust with phosphoric acid to a pH of 6.8, and dilute to 1 L.
Solution B: *Solution A* and water (1:24)
Mobile phase: Acetonitrile and *Solution B* (1:1). Adjust with phosphoric acid to a pH of 6.8.
Standard solution: (*L*/25) mg/mL of USP Drospirenone RS and of USP Ethinyl Estradiol RS in *Mobile phase*, where *L* is the Tablet label claim, in mg/Tablet, of each compound
Sample solution: Transfer 10 Tablets to a 250-mL volumetric flask, add 230 mL of *Mobile phase*, and sonicate with intermittent shaking for NLT 10 min, or until the Tablets are completely dispersed. Equilibrate to room temperature. Dilute with *Mobile phase* to volume, and centrifuge the sample until a clear supernatant is obtained. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector 1: UV 270 nm for drospirenone
Detector 2: Fluorescence, excitation wavelength at 285 nm, emission wavelength at 315 nm for ethinyl estradiol. [NOTE—*Detector 1* and *Detector 2* are connected in series.]
Column: 4.0-mm × 12.5-cm; 3-µm packing L1
Column temperature: 25 ± 3°
Flow rate: 1.2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.8 and 1.8 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 2.0% for both drospirenone and ethinyl estradiol

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of drospirenone ($C_{24}H_{30}O_3$) in the portion of Tablets taken:

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

r_U = peak response of drospirenone from the *Sample solution*
r_S = peak response of drospirenone from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of drospirenone in the *Sample solution* (mg/mL)

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

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

r_U = peak response of ethinyl estradiol from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of ethinyl estradiol; 90.0%–110.0% of drospirenone

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1: For drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol, or 3 mg of drospirenone and 0.02 mg of ethinyl estradiol
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Medium*, where *L* is the Tablet label claim of each compound. A volume of methanol not exceeding 2% of the final total volume of solution may be used to aid in dissolving these compounds.
Sample solution: Pass a portion of the solution under test through a suitable cellulose filter of 0.45-µm pore size, discarding the first 10 mL.
Mobile phase: Acetonitrile and water (40:60)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 210 nm and detection at 315 nm, or with excitation at 281 nm and detection at 305 nm
Column: 4.6-mm × 6-cm; 3-µm packing L1
Column temperature: 22°
Flow rate: 1 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 for both drospirenone and ethinyl estradiol
Tailing factor: Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 3% for both drospirenone and ethinyl estradiol
Samples: *Standard solution* and *Sample solution*
[NOTE—In *Medium*, drospirenone is partially converted into 17-epidrospirenone, which has a relative retention time of approximately 1.2 relative to drospirenone. The amount of drospirenone dissolved is calculated from the sum of drospirenone and 17-epidrospirenone.]
Calculate the percentage of drospirenone and ethinyl estradiol dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of drospirenone and NLT 75% (*Q*) of the labeled amount of ethinyl estradiol is dissolved.

Test 2: For drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol. If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Drospirenone RS and of USP Ethinyl Estradiol RS in *Medium*, where *L* is the Tablet label claim of each compound.
Sample solution: Centrifuge a portion of the solution under test at 3500 rpm for 15 min, and use the supernatant.
Mobile phase: Acetonitrile, methanol, and water (40:5:55)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 280 nm and detection at 310 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperature
Column: 30°
Autosampler: 5°
Flow rate: 1 mL/min
Injection size: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 3% for both drospirenone and ethinyl estradiol
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of drospirenone and ethinyl estradiol dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of drospirenone and NLT 85% (*Q*) of the labeled amount of ethinyl estradiol is dissolved.

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

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Acetonitrile, methanol, and water (26:19:55)
Solution B: Acetonitrile, methanol, and water (76:19:5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Flow (mL/min)	Solution A (%)	Solution B (%)
0	0.5	90	10
40	0.5	90	10
53	0.5	0	100
59	1.0	0	100
60	0.5	90	10
70	0.5	90	10

System suitability stock solution: ($L_1 \times 18/100$) µg/mL of USP Drospirenone RS in *Solution A*, where L_1 is the label claim (µg/Tablet) of drospirenone
System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* into a 10-mL volumetric flask, add 1.0 mL of 0.1 N HCl, and heat for 30 min in a 40° water bath. Immediately add 1 mL of 0.1 N NaOH and allow to reach room temperature. Dilute with *Solution A* to volume to obtain a solution containing drospirenone and 17-epidrospirenone. [NOTE—NaOH must be added immediately after heating for the reaction to proceed properly. The drospirenone to 17-epidrospirenone ratio must be between 3:1 and 7:1.]
Standard solution: ($L_1 \times 15/1000$) µg/mL of USP Drospirenone RS, ($L_2 \times 30/1000$) µg/mL of USP Ethinyl Estradiol RS, and ($L_2 \times 30/1000$) µg/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where L_1 and L_2 are the label claim (µg/Tablet) of drospirenone and ethinyl estradiol, respectively
Sensitivity solution: ($L_1 \times 15/10{,}000$) µg/mL of USP Drospirenone RS, ($L_2 \times 30/10{,}000$) µg/mL of USP Ethinyl Estradiol RS, and ($L_2 \times 30/10{,}000$) µg/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where L_1 and L_2 are the label claim (µg/Tablet) of drospirenone and ethinyl estradiol, respectively, prepared from the *Standard solution*
Sample solution: Transfer 15 Tablets to a 10-mL glass-stoppered test tube, and add 5.0 mL of *Solution A*. Shake vigorously, sonicate for NLT 5 min, and place in an ice bath for NLT 10 min. Centrifuge the sample at least until an almost clear supernatant is obtained. Filter the supernatant, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector 1: UV 222 nm
Detector 2: Fluorescence, excitation wavelength at 215 nm, emission wavelength at 315 nm. Monitor the signal at 344 nm between 37 and 42 min. [NOTE—*Detector 1* and *Detector 2* are connected in series. Use the response at 344 nm to quantify ethinyl estradiol related compound B.]
Column: 3.0-mm × 30-cm; 3-µm packing L1 followed, in series, by a 4.6-mm × 10-cm; chromolith packing L1
Column temperature: 40°
Flow rate: See *Table 1*.
Injection size: 20 µL
System suitability
Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Tailing Factor: Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol, *Standard solution*
Resolution: NLT 2.0 between drospirenone and 17-epidrospirenone, *System suitability solution*
Relative standard deviation: NMT 5.0% for both drospirenone and ethinyl estradiol, *Standard solution*
Signal-to-noise ratio: NLT 10 for drospirenone and ethinyl estradiol related compound B and NLT 7.0 for ethinyl estradiol, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the ethinyl estradiol degradation products using the relative retention times given in *Table 2*. Calculate the percentage of each ethinyl estradiol degradation product and unspecified degradation products 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 of each ethinyl estradiol degradation product from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (µg/mL)
F = relative response factor for each degradation product (see *Table 2*)

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethinyl estradiol related compound B in the portion of Tablets taken:

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

r_U = peak response of ethinyl estradiol related compound B from the *Sample solution*
r_S = peak response of ethinyl estradiol related compound B from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (µg/mL)

Samples: *Standard solution* and *Sample solution*
Identify the drospirenone degradation products using the relative retention times given in *Table 3*. Calculate the percentage of each drospirenone degradation product in the portion of Tablets taken:

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

r_U = peak response of each drospirenone degradation product from the *Sample solution*
r_S = peak response of drospirenone from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of drospirenone in the *Sample solution* (µg/mL)
F = relative response factor for each degradation product (see *Table 3*)

Acceptance criteria
[NOTE—Report only degradation products greater than 0.1%.]
Individual degradation products: See *Table 2* for ethinyl estradiol and *Table 3* for drospirenone.
Total degradation products: NMT 3.5%

Table 2

Name	Detection Mode	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)[a]	Acceptance Criteria, NMT (%)[b]
6α-Hydroxy ethinyl estradiol[c]	Fl (215 nm/315 nm)[g]	0 .25	0.73	0.3	0.3
6β-Hydroxy ethinyl estradiol[d]	Fl (215 nm/315 nm)	0.27	0.64	0.3	0.3
6-Keto ethinyl estradiol[e]	UV 222 nm	0.41	2.3	1.5	0.5
Ethinyl estradiol related compound B[f]	Fl (215 nm/344 nm)	0.88	—	1.0	1.0
Ethinyl estradiol	Fl (215 nm/315 nm)	1.0	—	—	—
Any unspecified degradation product	Fl (215 nm/315 nm) and UV 222[h]	—	1.0	0.3	0.5
Total degradation product	—	—	—	3.0	2.5

[a] Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.
[b] Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.
[c] 19-Nor-6α,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.
[d] 19-Nor-6β,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.
[e] 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol-6-one.
[f] Δ9,11-Ethinyl estradiol. 19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.
[g] Fl = Fluorescence.
[h] Determine unknown impurities using both modes of detection. Report the values from the detection mode that yield higher impurity levels.

Table 3

Name	Detection Mode (λ nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)[a]	Acceptance Criteria, NMT (%)[b]
Drospirenone	UV 222	0.75	—	—	—
17-Epidrospirenone[c]	UV 222	0.83	1.0	0.3	0.3
Ethinyl estradiol	UV 222	1.0	—	—	—
Any unspecified degradation product	UV 222	—	1.0	0.3	0.5
Total degradation product	—	—	—	0.5	1.0

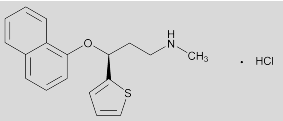
[a] Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.
[b] Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.
[c] 17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **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 Drospirenone RS
  USP Ethinyl Estradiol RS
  USP Ethinyl Estradiol Related Compound B RS
  19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.
  $C_{20}H_{22}O_2$ 294.39■2S (*USP35*)

Add the following:

■Duloxetine Hydrochloride



$C_{18}H_{19}NOS \cdot HCl$ 333.88
2-Thiophenepropanamine, *N*-methyl-γ-(1-naphthalenyloxy)-, hydrochloride, (*S*)-;
(+)-(*S*)-*N*-Methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [136434-34-9].

DEFINITION

Duloxetine Hydrochloride contains NLT 97.0% and NMT 102.0% of $C_{18}H_{19}NOS \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
  Sample solution: 5 mg/mL in methanol
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the duloxetine *S*-isomer from the *System suitability solution* in the test for *Limit of Duloxetine Related Compound A*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**
  Protect solutions of duloxetine from light.
  Buffer: 2.9 g/L of phosphoric acid in water. Adjust with sodium hydroxide solution to a pH of 2.5. To each L of this solution add 10.3 g of sodium 1-hexanesulfonate monohydrate, and dissolve.
  Mobile phase: Acetonitrile, *n*-propanol, and *Buffer* (13:17:70)
  Diluent: Acetonitrile and water (25:75)
  System suitability solution: 0.2 mg/mL of USP Duloxetine Hydrochloride RS in *Mobile phase*. Heat the solution to at least 40° for a minimum of 1 h. [NOTE—The resulting solution contains duloxetine impurity B, duloxetine impurity C, duloxetine impurity D, duloxetine impurity E, and duloxetine related compound F.]
  Standard solution: 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*
  Sample solution: 0.1 mg/mL of Duloxetine Hydrochloride in *Diluent*