Doxycycline Hyclate Delayed-Release Tablets

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Expert Committee: Small Molecules 1
Reason for Revision: Substantive error

In accordance with the Rules and Procedures of the 2020–2025 Council of Experts, the Small Molecules 1 Expert Committee has revised the Doxycycline Hyclate Delayed-Release Tablets monograph. The purpose for the revision is to address the inadvertent omission of Analysis and Tolerances sections from Dissolution Test 5 in the current official version as of May 01, 2019 to be consistent with the approved text in the previous version official as of August 01, 2017.

The Doxycycline Hyclate Delayed-Release Tablets Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Praveen K. Pabba, Scientific Liaison (301-816-8540 or pkp@usp.org).
Doxycycline Hyclate Delayed-Release Tablets

DEFINITION
Doxycycline Hyclate Delayed-Release Tablets contain an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline (C₂₂H₂₄N₂O₈).

IDENTIFICATION
• A. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
• B. The UV spectrum of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY
• Procedure
Protect solutions containing doxycycline from light.
Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.
Solution B: Methanol
Mobile phase: See Table 1.

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>2.0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>4.0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>6.0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>9.0</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Diluent: 0.01 N hydrochloric acid
Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in Diluent. Sonicate as needed to dissolve.
Sample solution: Nominally 0.1 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of Diluent, sonicate for about 15 min, shake for about 15 min, and dilute with Diluent to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.

Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 270 nm. For Identification B, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [Note—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 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 the labeled amount of doxycycline (C_{22}H_{24}N_{2}O_{8}) in the portion of Tablets taken:

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

\(r_U\) = peak response from the Sample solution
\(r_S\) = peak response from the Standard solution
\(C_S\) = concentration of USP Doxycycline Hyclate RS in the Standard solution (mg/mL)
\(C_U\) = nominal concentration of doxycycline in the Sample solution (mg/mL)
\(P\) = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
\(F\) = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution (711)**

Protect solutions containing doxycycline from light.

Test 1: Proceed as directed for Dissolution (711), Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL, degassed with helium

Apparatus 1: 50 rpm

Time: 20 min

Standard solution: 0.128 mg/mL of USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [Note—Sonicate if necessary to dissolve.]

Sample solution: Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

Detector: UV 346 nm

Cell: 0.1-cm quartz

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of doxycycline (C_{22}H_{24}N_{2}O_{8}) dissolved:

\[
\text{Result} = \left( \frac{A_U}{A_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]
\[ A_U = \text{absorbance of the Sample solution} \]
\[ A_S = \text{absorbance of the Standard solution} \]
\[ C_S = \text{concentration of doxycycline in the Standard solution (mg/mL)} \]
\[ L = \text{label claim (mg/Tablet)} \]
\[ V = \text{volume of Medium, 900 mL} \]

**Tolerances**

**Level 1** (6 Tablets tested): No individual value is more than 30% of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved in 20 min.

**Level 2** (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) in 20 min.

**Buffer stage**

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the Acid stage testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see Reagents, Indicators, and Solutions—Solutions, Buffer Solutions); 900 mL, degassed

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Standard solution:** 0.128 mg/mL of USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [Note—Sonicate if necessary to dissolve.]

**Sample solution:** Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

**Analysis:** Determine the percentage of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved by the procedure described for the Acid stage.

**Tolerances:** NLT 85% \((Q)\) of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP Dissolution Test 2. Proceed as directed for Dissolution (711), Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.

**Acid stage**

**Medium, Apparatus 1, Time, Blank, and Analysis:** Proceed as directed for Acid stage in Test 1.

**Standard solution:** \((L/900)\) mg/mL of USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

**Sample solution:** Pass portions of the solution under test through a suitable filter.

**Detector:** UV 345 nm

**Cell:** See Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Tablet Strength (mg/Tablet)</th>
<th>Cell Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.5</td>
</tr>
<tr>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>150</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Tolerances

Level 1 (6 Tablets tested): No individual value is more than 50% of the labeled amount of doxycycline \((C_{22}H_{24}N_{2}O_{8})\) dissolved in 20 min.

Level 2 (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 50% of the labeled amount of doxycycline \((C_{22}H_{24}N_{2}O_{8})\) in 20 min.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the Acid stage testing.

Medium: pH 5.5 neutralized phthalate buffer (see Reagents, Indicators, and Solutions—Solutions, Buffer Solutions); 1000 mL, degassed

Apparatus 1 and Analysis: Proceed as directed for Buffer stage in Test 1.

Time: 45 min

Standard solution: \((L/1000)\) mg/mL of USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector and Cell: Proceed as directed for Acid stage in Test 2.

Tolerances: NLT 70% \((Q)\) of the labeled amount of doxycycline \((C_{22}H_{24}N_{2}O_{8})\) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP Dissolution Test 3. Proceed as directed for Dissolution (711), Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.

Acid stage

Apparatus 1 and Time: Proceed as directed for Acid stage in Test 1.

Medium: 0.06 N hydrochloric acid; 900 mL

Standard solution: Prepare the solutions from USP Doxycycline Hyclate RS in Medium as directed in Table 3. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

<table>
<thead>
<tr>
<th>Tablet Strength (mg/Tablet)</th>
<th>Concentration of Doxycycline (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.1</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>150</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2 cm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of doxycycline \((C_{22}H_{24}N_{2}O_{8})\) dissolved:

\[
\text{Result} = \left( \frac{A_U}{A_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]
\[ A_U = \text{absorbance of the Sample solution} \]
\[ A_S = \text{absorbance of the Standard solution} \]
\[ C_S = \text{concentration of doxycycline in the Standard solution (mg/mL)} \]
\[ L = \text{label claim (mg/Tablet)} \]
\[ V = \text{volume of Medium, 900 mL} \]

**Tolerances:** See Table 4.

### Table 4

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of Tablets Tested</th>
<th>Tablets Labeled to Contain 75 or 100 mg of Doxycycline</th>
<th>Tablets Labeled to Contain 150 mg of Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_1 )</td>
<td>6</td>
<td>No individual value exceeds 50% at 20 min.</td>
<td>No individual value exceeds 30% at 20 min.</td>
</tr>
<tr>
<td>( A_2 )</td>
<td>6</td>
<td>Average of 12 units ( (A_1 + A_2) ) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.</td>
<td>Average of 12 units ( (A_1 + A_2) ) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.</td>
</tr>
<tr>
<td>( A_3 )</td>
<td>12</td>
<td>Average of 24 units ( (A_1 + A_2 + A_3) ) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.</td>
<td>Average of 24 units ( (A_1 + A_2 + A_3) ) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.</td>
</tr>
</tbody>
</table>

**Buffer stage**

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the Acid stage testing.

**Medium:** pH 5.5 neutralized phthalate buffer (see Reagents, Indicators, and Solutions—Solutions, Buffer Solutions); 1000 mL

**Apparatus 1:** 50 rpm

**Time:** 60 min

**Standard solution:** Prepare the solutions from USP Doxycycline Hyclate RS in Medium as directed in Table 5. Calculate the concentration, \( C_{S'} \), in mg/mL, of doxycycline using the designated potency, in \( \mu g/mg \), of doxycycline in USP Doxycycline Hyclate RS.

### Table 5

<table>
<thead>
<tr>
<th>Tablet Strength (mg/Tablet)</th>
<th>Concentration of Doxycycline (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.1</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>150</td>
<td>0.15</td>
</tr>
</tbody>
</table>
**Sample solution**: Pass portions of the solution under test through a suitable filter.

**Detector**: UV 345 nm

**Cell**: 0.2 cm

**Blank**: Medium

**Analysis**

**Samples**: Standard solution and Sample solution

Calculate the percentage of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved:

\[
\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100
\]

- \(A_U\) = absorbance of the Sample solution
- \(A_S\) = absorbance of the Standard solution
- \(C_S\) = concentration of doxycycline in the Standard solution (mg/mL)
- \(L\) = label claim (mg/Tablet)
- \(V\) = volume of Medium, 1000 mL

**Tolerances**: See *Table 6*.

**Table 6**

<table>
<thead>
<tr>
<th>Tablets Labeled to Contain 75 or 100 mg of Doxycycline</th>
<th>Tablets Labeled to Contain 150 mg of Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLT 80% ((Q)) of the labeled amount of doxycycline ((C_{22}H_{24}N_2O_8)) is dissolved.</td>
<td>NLT 70% ((Q)) of the labeled amount of doxycycline ((C_{22}H_{24}N_2O_8)) is dissolved.</td>
</tr>
</tbody>
</table>

**Test 4**: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*. Proceed as directed for *Dissolution (711), Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

**Acid stage**

- **Medium**: 0.06 N hydrochloric acid; 900 mL, degassed
- **Apparatus 1**: 50 rpm
- **Time**: 20 min

**Standard solution**: 0.1 mg/mL of doxycycline from *USP Doxycycline Hyclate RS* in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in *USP Doxycycline Hyclate RS*.

**Sample solution**: Pass portions of the solution under test through a suitable filter.

**Detector**: UV 345 nm

**Cell**: 0.2-cm quartz

**Blank**: Medium

**Analysis**

**Samples**: Standard solution and Sample solution

Calculate the percentage of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved:

\[
\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100
\]

- \(A_U\) = absorbance of the Sample solution
\(A_S\) = absorbance of the Standard solution

\(C_S\) = concentration of doxycycline in the Standard solution (mg/mL)

\(L\) = label claim (mg/Tablet)

\(V\) = volume of Medium, 900 mL

**Tolerances**

**Level 1** (6 Tablets tested): No individual value is more than 30% of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved in 20 min.

**Level 2** (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved in 20 min.

**Buffer stage**

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the Acid stage testing.

**Medium**: pH 5.5 neutralized phthalate buffer (see Reagents, Indicators, and Solutions—Solutions, Buffer Solutions); 1000 mL, degassed

**Apparatus 1**: 50 rpm

**Time**: 30 min

**Standard solution**: 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in Medium

**Sample solution**: Pass portions of the solution under test through a suitable filter. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in \(\mu\text{g/mg}\), of doxycycline in USP Doxycycline Hyclate RS.

**Blank**: Medium

**Analysis**

**Samples**: Standard solution and Sample solution

Calculate the percentage of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) dissolved:

\[
\text{Result} = \left(\frac{A_U}{A_S}\right) \times \left(\frac{C_S}{L}\right) \times V \times 100
\]

\(A_U\) = absorbance of the Sample solution

\(A_S\) = absorbance of the Standard solution

\(C_S\) = concentration of doxycycline in the Standard solution (mg/mL)

\(L\) = label claim (mg/Tablet)

\(V\) = volume of Medium, 1000 mL

**Tolerances**: NLT 75% (Q) of the labeled amount of doxycycline \((C_{22}H_{24}N_2O_8)\) is dissolved.

**Test 5**: If the product complies with this test, the labeling indicates that the product meets USP Dissolution Test 5. Proceed as directed for Dissolution (711), Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.

**Acid stage**

**Medium**: 0.06 N hydrochloric acid; 900 mL

**Apparatus 1**: 100 rpm

**Time**: 20 min

**Standard solution**: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in \(\mu\text{g/mg}\), of doxycycline in USP Doxycycline Hyclate RS.

**Sample solution**: Pass portions of the solution under test through a suitable filter.
Detector: UV 345 nm  
Cell: 1.0 cm  
Blank: Medium  

Analysis  
Samples: Standard solution and Sample solution  

Calculate the percentage of the labeled amount of doxycycline (C_{22}H_{24}N_{2}O_{8}) dissolved:

\[
\text{Result} = \left( \frac{A_U}{A_S} \right) \times \left( \frac{C_S}{L} \right) \times V \times 100
\]

- \(A_U\) = absorbance of the Sample solution  
- \(A_S\) = absorbance of the Standard solution  
- \(C_S\) = concentration of doxycycline in the Standard solution (mg/mL)  
- \(L\) = label claim (mg/Tablet)  
- \(V\) = volume of Medium, 900 mL  

Tolerances: See Table 7.

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of Tablets Tested</th>
<th>Tolerances</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_1)</td>
<td>6</td>
<td>No individual value exceeds 50% at 20 min.</td>
</tr>
<tr>
<td>(A_2)</td>
<td>6</td>
<td>Average of 12 units ((A_1 + A_2)) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.</td>
</tr>
<tr>
<td>(A_3)</td>
<td>12</td>
<td>Average of 24 units ((A_1 + A_2 + A_3)) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.</td>
</tr>
</tbody>
</table>

Buffer stage  
Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the Acid stage testing.  
Medium: pH 5.5 neutralized phthalate buffer (see Reagents, Indicators, and Solutions—Solutions, Buffer Solutions); 900 mL  
Apparatus 1: 100 rpm  
Time: 30 min  
Standard solution: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in Medium. Calculate the concentration, \(C_S\), in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.  
Sample solution: Pass portions of the solution under test through a suitable filter.  
Blank: Medium  

\[\text{^\textcircled{A}}\text{Analysis}\]  
Samples: Standard solution and Sample solution  

Calculate the percentage of the labeled amount of doxycycline (C_{22}H_{24}N_{2}O_{8}) dissolved:
Result = \( \frac{A_U}{A_S} \times \frac{C_S}{L} \times V \times 100 \)

- \( A_U \): absorbance of the Sample solution
- \( A_S \): absorbance of the Standard solution
- \( C_S \): concentration of doxycycline in 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 doxycycline \( (C_{22}H_{24}N_2O_8) \) is dissolved. (RB 1-Oct-2020)

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

**Impurities**

- **Organic Impurities:**
  Protect solutions containing doxycycline from light.

**Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability stock solution 1:** 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in Diluent

**System suitability stock solution 2:** 1.2 mg/mL of USP Doxycycline Hyclate RS in Diluent

**System suitability solution:** Transfer 5 mL of System suitability stock solution 2 to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in Diluent, add 0.5 mL of System suitability stock solution 1, and dilute with Diluent to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [Note—The solution is stable up to 14 days when stored in a refrigerator.]

**Sensitivity solution:** 2 µg/mL of USP Doxycycline Hyclate RS in Diluent

**Standard solution:** 4.6 µg/mL of USP Doxycycline Hyclate RS in Diluent

**Sample solution:** Nominally 2.0 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of Diluent, sonicate for about 15 min, shake for about 15 min, and dilute with Diluent to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.

**System suitability**

- **Samples:** System suitability solution, Sensitivity solution, and Standard solution
- **Suitability requirements**
  - **Resolution:** NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, System suitability solution
  - **Relative standard deviation:** NMT 5.0% for the doxycycline peak, Standard solution
  - **Signal-to-noise ratio:** NLT 10, Sensitivity solution

**Analysis**

- **Samples:** Standard solution and Sample solution
  Calculate the percentage of each impurity in the portion of Tablets taken:

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

- \( r_U \): peak response of each impurity from the Sample solution
- \( r_S \): peak response of doxycycline from the Standard solution
- \( C_S \): concentration of USP Doxycycline Hyclate RS in the Standard solution (mg/mL)

C280855-M2243-SM12020, rev. 00 20200925
\[ C_U \] = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

\[ P \] = potency of doxycycline in **USP Doxycycline Hyclate RS** (µg/mg)

\[ F \] = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 8*. The reporting threshold is 0.1%.

**Table 8**

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline(a,\ b)</td>
<td>0.39</td>
<td>—</td>
</tr>
<tr>
<td>Methacycline(b,\ c)</td>
<td>0.64</td>
<td>—</td>
</tr>
<tr>
<td>4-Epidoxycycline(d)</td>
<td>0.79</td>
<td>1.0</td>
</tr>
<tr>
<td>Doxycycline related compound A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6-epidoxycycline)(b,\ e)</td>
<td>0.88</td>
<td>—</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(a\) (4S,4\(R\),5\(S\),5a\(R\),6\(S\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydoxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

\(b\) Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

\(c\) (4S,4\(R\),5\(S\),5a\(R\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydoxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

\(d\) (4\(R\),4\(R\),5\(S\),5a\(R\),6\(R\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydoxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Main degradation product.

\(e\) (4S,4\(R\),5\(S\),5a\(R\),6\(S\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydoxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **Labeling:** When more than one Dissolution test is given, the labeling states the test used only if Test 1 is not used.
- **USP Reference Standards (11)**
  - **USP Doxycycline Hyclate RS**
  - **USP Doxycycline Related Compound A RS**

  [Note—May be available as a free base or a hydrochloride salt.]

6-Epidoxycycline, or (4\(S\),4\(R\),5\(S\),5a\(R\),6\(S\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydoxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

\[ C_{22}H_{24}N_2O_8 \] 444.43

(4\(S\),4\(R\),5\(S\),5a\(R\),6\(S\),12a\(S\))-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydoxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.

\[ C_{22}H_{24}N_2O_8 \cdot HCl \] 480.13

**USP Methacycline Hydrochloride RS**