Doxorubicin Hydrochloride Injection

DEFINITION
Doxorubicin Hydrochloride Injection is a sterile solution of Doxorubicin Hydrochloride in Sterile Water for Injection made isosmotic with Sodium Chloride, Dextrose, or other suitable added substances. It contains NLT 90.0% and NMT 115.0% of the labeled amount of doxorubicin hydrochloride (C27H29NO11 · HCl).

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

ASSAY

**Change to read:**

- **PROCEDURE**
  - [NOTE—Protect solutions containing doxorubicin from light.]
  - **Solution A:** 0.1% trifluoroacetic acid TS
  - **Solution B:** Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)
  - **Mobile phase:** See Table 1.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>16.1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>18</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Diluent: Solution A and Solution B (50:50)
System suitability solution: 0.1 mg/mL each of USP Doxorubicin Hydrochloride RS and USP Epirubicin Hydrochloride RS in Diluent
Standard solution: 0.1 mg/mL of USP Doxorubicin Hydrochloride RS in Diluent
Sample solution: Nominally 0.1 mg/mL of doxorubicin hydrochloride in Diluent from Injection

**Chromatographic system**
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 254 nm. For Identification B, use a diode array detector in the range of 190–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Temperature: Autosampler: 4°C
Column: 35°C
Flow rate: 0.5 mL/min
Injection volume: 2 µL
System suitability
- **Samples:** System suitability solution and Standard solution
- [NOTE—See Table 2 for the relative retention times.]
- **Suitability requirements**
  - Resolution: NLT 1.5 between doxorubicin and epirodarubicin, System suitability solution
  - Tailing factor: 0.8–1.5, Standard solution
  - Relative standard deviation: NMT 0.73%, Standard solution

**Organic Impurities**
[NOTE—Protect solutions containing doxorubicin from light.]
Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.
Standard solution: 0.008 mg/mL each of USP Doxorubicin Hydrochloride RS and 0.012 mg/mL of USP Epirubicinone RS in Diluent. [NOTE—It may be necessary to first dissolve in acetonitrile, using NMT 5% of the final volume, before diluting with Diluent.]  
Sample solution: Nominally 0.4 mg/mL of doxorubicin hydrochloride in Diluent from Injection

**System suitability**
- **Samples:** System suitability solution and Standard solution
- **Suitability requirements**
  - Resolution: NLT 1.5 between doxorubicin and epirodarubicin, System suitability solution
  - Relative standard deviation: NMT 5.0%, Standard solution

**Analysis**
**Samples:** Standard solution and Sample solution
Calculate the percentage of the labeled amount of doxorubicin hydrochloride (C27H29NO11 · HCl) in the portion of Injection 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 doxorubicin from the Sample solution
- **r_S** = peak response of doxorubicin from the Standard solution
- **C_S** = concentration of USP Doxorubicin Hydrochloride RS in the Standard solution (mg/mL)
- **C_U** = nominal concentration of doxorubicin hydrochloride in the Sample solution (mg/mL)
- **P** = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)
- **F** = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

**Impurities**

**Analysis**
**Samples:** Standard solution and Sample solution
Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

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

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Doxorubicin

\( r_0 \) = peak response of each degradation product from the Sample solution
\( r_S \) = peak response of doxorubicin from the Sample solution
\( C_S \) = concentration of USP Doxorubicin Hydrochloride RS in the Standard solution (mg/mL)
\( C_U \) = nominal concentration of doxorubicin hydrochloride in the Sample solution (mg/mL)
\( P \) = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)
\( F \) = conversion factor, 0.001 mg/µg

Acceptance criteria: See Table 2.

**SPECIFIC TESTS**
- \( pH \) (791): 2.5—4.5
- **Sterility Tests** (71), Test for Sterility of the Product to Be Examined, Membrane Filtration: It meets the requirements when tested as directed, the entire contents of all the containers being collected aseptically.
- **Bacterial Endotoxins Test** (85)
  - Sample solution: 1.1 mg/mL of doxorubicin hydrochloride prepared from Injection in Sterile Water for Injection
  - Acceptance criteria: NMT 2.2 USP Endotoxin Units/mg of doxorubicin hydrochloride
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store in a refrigerator. Injection may be packaged in multiple-dose containers not exceeding 100 mL in volume.

**Change to read:**

*USP Reference Standards* (11)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Epirubicine</td>
<td>1.05</td>
<td>—</td>
</tr>
<tr>
<td>Doxorubicinone</td>
<td>1.08</td>
<td>3.0</td>
</tr>
<tr>
<td>Daunorubicinone</td>
<td>1.35</td>
<td>—</td>
</tr>
<tr>
<td>Any other individual degradation product</td>
<td>—</td>
<td>2.0</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*For resolution measurement only. Not to be reported; not to be included in total impurities;* 
*\((85,10S)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.\)*
*\((85,10S)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.\)*
*The acceptance criteria of this impurity, if present, would fall under the acceptance criteria for “any other individual degradation product” and is included in the total impurities.*

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