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Doxorubicin Hydrochloride for Injection

DEFINITION

Doxorubicin Hydrochloride for Injection is a sterile mixture of Doxorubicin Hydrochloride and Lactose. It contains NLT 90.0% and NMT 115.0% of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$).

[Caution—Great care should be taken to prevent inhaling particles of Doxorubicin Hydrochloride and exposing the skin to it.]

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**

Solution A: 0.1% Trifluoroacetic acid prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

Solution B: Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	25	75
16	25	75
16.1	90	10
18	90	10

Diluent: *Solution A* and *Solution B* (50:50)

[Note—Protect solutions containing doxorubicin from light.]

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 Doxorubicin Hydrochloride for Injection prepared as follows. Add 5 mL of *Diluent* into the container of Doxorubicin Hydrochloride for Injection, and transfer the contents to a volumetric flask of appropriate size. Rinse the container with additional *Diluent* NLT 3 times. Dilute with *Diluent* to volume, and mix.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm. For *Identification* test *B*, use a photo-diode array detector in the range of 190–400 nm.

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Temperatures

Autosampler: 4°

Column: 35°

Flow rate: 0.5 mL/min

Injection volume: 2 µL

System suitability

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

[Note—The relative retention times for doxorubicin and epirubicin are 1.0 and 1.05, respectively.]

Suitability requirements

Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Doxorubicin Hydrochloride for Injection taken:

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

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 ▲hydrochloride▲ (ERR 1-Nov-2022) in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

IMPURITIES

Change to read:

• **Organic Impurities**

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

[Note—Protect solutions containing doxorubicin from light.]

Standard solution: 0.002 mg/mL each of USP Doxorubicin Hydrochloride RS, USP Doxorubicinone RS, and USP Daunorubicinone RS in *Diluent*

Sample solution: Nominally 0.4 mg/mL of doxorubicin hydrochloride in *Diluent* from Doxorubicin Hydrochloride for Injection prepared as follows. Add 5 mL of *Diluent* into the container of Doxorubicin Hydrochloride for Injection, and transfer the contents to a volumetric flask of appropriate size. Rinse the container with additional *Diluent* NLT 3 times. Dilute with *Diluent* to volume, and mix.

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 epirubicin, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxorubicinone in the portion of Doxorubicin Hydrochloride for Injection taken:

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

r_U = peak response of doxorubicinone from the *Sample solution*

r_S = peak response of doxorubicinone from the *Standard solution*

C_S = concentration of USP Doxorubicinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicinone in USP Doxorubicinone RS (mg/mg)

Calculate the percentage of daunorubicinone in the portion of Doxorubicin Hydrochloride for Injection taken:

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

r_U = peak response of daunorubicinone from the *Sample solution*

r_S = peak response of daunorubicinone from the *Standard solution*

C_S = concentration of USP Daunorubicinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of daunorubicinone in USP Daunorubicinone RS (mg/mg)

Calculate the percentage of any individual unspecified degradation product in the portion of Doxorubicin Hydrochloride for Injection taken:

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

r_U = peak response of each unspecified degradation product 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 ▲hydrochloride▲ (ERR 1-Nov-2022) in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxorubicin	1.0	—
Epirubicin[a]	1.05	—
Doxorubicinone[b]	1.08	0.5
Daunorubicinone[c]	1.35	0.5
Any individual unspecified degradation product	—	0.5
Total impurities	—	2.0

[a] For resolution measurement only. Not to be reported; not to be included in total impurities.

[b] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

[c] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

SPECIFIC TESTS

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products ⟨1⟩, Specific Tests, Completeness and clarity of solutions*.

• **Sterility Tests ⟨71⟩:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*, the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.

• **Water Determination, *Method I*⟨921⟩**

Sample solution: Prepare as directed for a hygroscopic specimen.

Acceptance criteria: NMT 4.0%

• **pH ⟨791⟩**

Sample solution: Constitute as directed in the labeling using water as the diluent.

Acceptance criteria: 4.5–6.5

• **Bacterial Endotoxins Test ⟨85⟩**

Sample solution: 1.1 mg/mL of doxorubicin hydrochloride from Doxorubicin Hydrochloride for Injection

Acceptance criteria: NMT 2.2 USP Endotoxin Units/mg of doxorubicin hydrochloride

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

• **Labeling ⟨7⟩, Labels and Labeling for Injectable Products:** Meets the requirements.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution*, except that multiple-dose containers may provide for the withdrawal of NMT 100 mL when constituted as directed in the labeling. Store unreconstituted vials at controlled room temperature. Protect from light.

• **USP Reference Standards ⟨11⟩**

USP Daunorubicinone RS

(8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

$C_{21}H_{18}O_8$ 398.36

USP Doxorubicin Hydrochloride RS

USP Doxorubicinone RS

(8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

$C_{21}H_{18}O_9$ 414.36

USP Epirubicin Hydrochloride RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
DOXORUBICIN HYDROCHLORIDE FOR INJECTION	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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