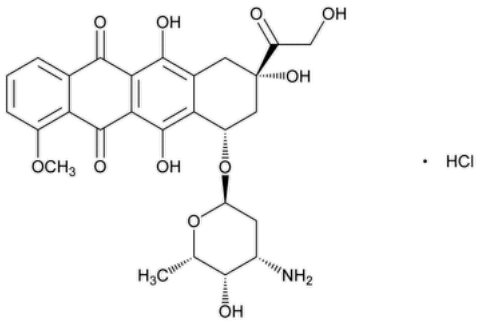
Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-May-2024
Document Type: USP Monographs
DocId: GUID-F2F8F379-32A6-431D-A217-105FDE9DAC07_5_en-US
DOI: https://doi.org/10.31003/USPNF_M28180_05_01
DOI Ref: v200r

Doxorubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$ 579.98

5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxylacetyl)-1-methoxy-, hydrochloride (8*S*-*cis*)-;
(8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride CAS RN®: 25316-40-9; UNII: 82F2G7BL4E.

DEFINITION

Doxorubicin Hydrochloride contains NLT 98.0% and NMT 102.0% of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$), calculated on the anhydrous, solvent-free basis.

[**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.** **Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**
- **C.** **Identification Tests—General,** *Chloride* ⟨191⟩

ASSAY

Change to read:

- **Procedure**

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

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▲ (ERR 1-May-2024) in *Diluent*

Standard solution: 0.1 mg/mL of USP Doxorubicin Hydrochloride RS in *Diluent*

Sample solution: 0.1 mg/mL of Doxorubicin Hydrochloride in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Temperatures

Column: 35°

Autosampler: 4°

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 doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Doxorubicin Hydrochloride 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 = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicin hydrochloride in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

• **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, USP Daunorubicin Hydrochloride RS, and USP Daunorubicinone RS in *Diluent*

Sample solution: 0.4 mg/mL of Doxorubicin Hydrochloride in *Diluent*

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 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 = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

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

Calculate the percentage of daunorubicinone in the portion of Doxorubicin Hydrochloride 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 = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

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

Calculate the percentage of daunorubicin in the portion of Doxorubicin Hydrochloride taken:

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

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

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

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

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of daunorubicin in USP Daunorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of any individual unspecified impurity in the portion of Doxorubicin Hydrochloride taken:

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

r_U = peak response of any individual unspecified impurity 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 = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicin hydrochloride 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
Daunorubicin	1.23	0.5
Daunorubicinone[c]	1.35	0.5
Any individual unspecified impurity	—	0.5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
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-Tetrahydoxy-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.

• **Limit of Acetone and Alcohol**

Internal standard solution: 1 mg/mL of dioxane in water

Standard solution: 0.2 mg/mL of USP Acetone RS, 0.3 mg/mL of dehydrated alcohol in *Internal standard solution*

Sample solution: 200 mg of Doxorubicin Hydrochloride in 3.0 mL (3.0 g) of *Internal standard solution*

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 4-mm × 2-m; packed with 8%–10% liquid phase G16 and 2% potassium hydroxide on 100- to 120-mesh support S1A

Column temperature: 60°

Carrier gas: Helium

[Note—Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 min.]

Injection volume: 1 µL

Flow rate: Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 min.

System suitability

Sample: *Standard solution*

[Note—The relative retention times for acetone, alcohol, and dioxane are about 0.2, 0.5, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between adjacent peaks

Tailing factor: NMT 1.5 for the alcohol peak

Relative standard deviation: NMT 4.0% for the peak response ratios of acetone and alcohol to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage, by weight, of acetone and alcohol respectively, in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_A/C_D) \times (D_U/W_U) \times 100$$

R_U = peak response ratio of the analyte (acetone or alcohol) to dioxane from the *Sample solution*

R_S = peak response ratio of the analyte (acetone or alcohol) to dioxane from the *Standard solution*

C_A = concentration of the analyte (acetone or alcohol) in the *Standard solution* (mg/mL)

C_D = concentration of dioxane in the *Standard solution* (mg/mL)

D_U = weight of dioxane in the *Sample solution* (mg)

W_U = weight of Doxorubicin Hydrochloride in the *Sample solution* (mg)

Acceptance criteria

Acetone: NMT 0.5%

Total of acetone and alcohol: NMT 2.5%

SPECIFIC TESTS

• **Crystallinity ⟨695⟩:** Meets the requirements, except where it is labeled as amorphous, most particles do not exhibit birefringence and extinction positions

• **pH ⟨791⟩**

Sample solution: 5 mg/mL

Acceptance criteria: 4.0–5.5

• **Water Determination, *Method I* ⟨921⟩:** NMT 4.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature except where it is labeled as amorphous, in which case it should be stored in the freezer.

• **Labeling:** The amorphous form is so labeled.

- **USP Reference Standards** ⟨11⟩

USP Acetone RS

USP Daunorubicin Hydrochloride RS

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	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(3)

Current DocID: GUID-F2F8F379-32A6-431D-A217-105FDE9DAC07_5_en-US

DOI: https://doi.org/10.31003/USPNF_M28180_05_01

DOI ref: v200r