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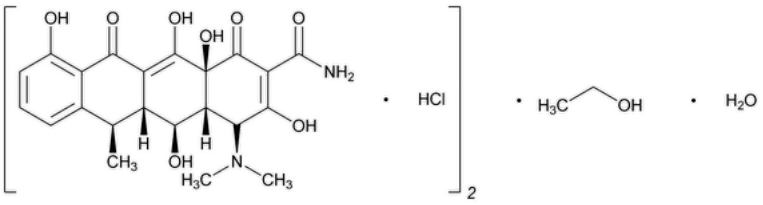
Doxycycline Hyclate

Change to read:



$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$ ▲1025.88▲ (ERR 1-Jun-2022)

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, compound with ethanol (2:1), monohydrate, [4*S*-(4α,4aα,5α,5aα,6α,12aα)]-;
(4*S*,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 monohydrochloride, compound with ethyl alcohol (2:1), monohydrate CAS RN®: 24390-14-5; UNII: 19XTS3T51U.

DEFINITION

Doxycycline Hyclate has a potency equivalent to NLT 800 µg/mg and NMT 920 µg/mg of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Chloride*:** Meets the requirements

ASSAY

- **Procedure**

Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate and 0.5 g of edetate disodium to a 1000-mL volumetric flask. Add about 850 mL of water and 0.5 mL of triethylamine, and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2.0	90	10
6.0	85	15
8.0	60	40
8.1	90	10
10.0	90	10

Diluent: 0.01 N hydrochloric acid

Standard solution: 0.1 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: 0.1 mg/mL of Doxycycline Hyclate in *Diluent*. Sonicate as needed to dissolve.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

Column: 2.1-mm × 5-cm; 1.7-µm packing L7

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 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of doxycycline ($C_{22}H_{24}N_2O_8$), in µg/mg, in the portion of Doxycycline Hyclate taken:

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

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 = concentration of Doxycycline Hyclate in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

Acceptance criteria: 800–920 µg/mg

IMPURITIES

Change to read:

• **Organic Impurities**

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

System suitability stock solution 1: 1 mg/mL each of USP Methacycline Hydrochloride RS and USP Doxycycline Related Compound A 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, methacycline, doxycycline related compound A, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Sensitivity solution: 0.001 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

Standard solution: 0.002 mg/mL each of USP Doxycycline Hyclate RS and USP Methacycline Hydrochloride RS in *Diluent*

Sample solution: 2 mg/mL of Doxycycline Hyclate in *Diluent*. Sonicate as needed to dissolve.

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% each for the doxycycline and methacycline peaks, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methacycline in the portion of Doxycycline Hyclate taken:

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

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

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

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

C_U = concentration of Doxycycline Hyclate in the *Sample solution* (mg/mL)

P = potency of methacycline in USP Methacycline Hydrochloride RS (µg/mL)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of 4-epidoxycycline, doxycycline related compound A, doxycycline related compound F, and any individual unspecified impurity in the portion of Doxycycline Hyclate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

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)

C_U = concentration of Doxycycline Hyclate in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methacycline	0.51	1.0	2.0
4-Epidoxycycline[a]	0.62	1.0	0.5
Doxycycline related compound A (6-epidoxycycline)	0.72	0.60	2.0
Doxycycline	1.0	—	—
Doxycycline related compound F[b]	1.20	0.61	1.0
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.5

[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. Main degradation product.

▲[b] (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-2-Acetyl-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione.▲ (ERR 1-Jun-2022)

• **Content of Ethanol**

Diluent: Dimethylacetamide

Standard solution: 2.5 mg/mL of ethanol in *Diluent*

Sample solution: 50 mg/mL of Doxycycline Hyclate in *Diluent* prepared as follows. Transfer 100 mg of Doxycycline Hyclate to a 10-mL flask, fitted with a septum, add 2 mL of *Diluent*, and vortex to dissolve.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Columns

Guard: 1-m deactivated silica

Analytical: 0.32-mm × 30-m fused silica; coated with a 1.0-µm film of phase G25

Temperatures

Injection port: 150°

Detector: 250°

Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
45	—	45	2
45	15	100	—
100	40	240	5

Carrier gas: Helium

Flow rate: 5 mL/min

Injection volume: 1 µL

Injection type: Split; split ratio 6:1

Run time: NLT 2.2 times the retention time of ethanol

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.2

Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ethanol in the portion of Doxycycline Hyclate taken:

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

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

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

C_S = concentration of ethanol in the *Standard solution* (mg/mL)

C_U = concentration of Doxycycline Hyclate in the *Sample solution* (mg/mL)

Acceptance criteria: 4.3%–5.5%; for veterinary products, 4.3%–6.0%

SPECIFIC TESTS

• **Crystallinity ⟨695⟩:** Meets the requirements

• **pH ⟨791⟩**

Sample solution: Nominally 10 mg/mL of doxycycline from Doxycycline Hyclate

Acceptance criteria: 2.0–3.0

• **Water Determination ⟨921⟩, *Method I*:** 1.4%–2.8%

• **Sterility Tests ⟨71⟩:** Where the label states that Doxycycline Hyclate is sterile, it meets the requirements. If the membrane filtration test is used, use *Fluid D* instead of *Fluid A*.

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Doxycycline Hyclate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it has NMT 1.14 USP Endotoxin Units/mg of doxycycline.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Change to read:

• **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.]

(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.

$C_{22}H_{24}N_2O_8$ ▲444.44▲ (ERR 1-Jun-2022)

(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, monohydrochloride. $C_{22}H_{24}N_2O_8 \cdot HCl$ ▲480.90▲ (ERR 1-Jun-2022)

USP Methacycline Hydrochloride RS

(4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide hydrochloride.

$C_{22}H_{22}N_2O_8 \cdot HCl$ 478.88

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

Topic/Question	Contact	Expert Committee
DOXYCYCLINE HYCLATE	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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